ASSESSMENT OF PROTEINURIA BY URINE SPOT PROTEIN CREATININE RATIO FOR RISK PREDICTION OF DENGUE HEMORRHAGIC FEVER/DENGUE SHOCK SYNDROME IN DENGUE INFECTIONS

Dissertation submitted in partial fulfillment of the

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DOCTOR OF MEDICINE

BRANCH I - GENERAL MEDICINE

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CHENNAI, TAMILNADU

CERTIFICATE FROM THE DEAN

This is to certify that the dissertation entitled "**ASSESSMENT OF PROTEINURIA BY URINE SPOT PROTEIN CREATININE RATIO FOR RISK PREDICTION OF DENGUE HEMORRHAGIC FEVER/DENGUE SHOCK SYNDROME IN DENGUE INFECTIONS**" is the bonafide work of **Dr. R.DINESH** in partial fulfillment of the university regulations of the Tamil Nadu Dr. M.G.R. Medical University, Chennai, for **M.D General Medicine Branch I** examination to be held in April 2019.

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DECLARATION

I, **Dr.R.Dinesh** declare that, I carried out this work on "**Assessment of proteinuria by urine spot protein creatinine ratio for risk prediction of dengue hemorrhagic fever/dengue shock syndrome in dengue infections**" at the Department of General Medicine, Government Rajaji Hospital, Madurai under the guidance of **Dr. J.SANGUMANI M.D.,** Professor, Department of General Medicine, Madurai medical College, Madurai.

I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree, Diploma to any other University, Board either in India or abroad.

This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the rules and regulations for the award of **Doctor of Medicine (M.D.), General Medicine Branch-I**, examination to be held in **April 2019.**

Place: Madurai
Date:

Dr. R.DINESH

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CONTENTS

INTRODUCTION

Dengue fever is caused by flavi virus and is spread by aedes mosquito mostly aedes aegypti. It has a mean incubation period of 2-5 days and has been seen to occur as seasonal outbreaks.

Dengue fever can occur with varied spectrum of presentations. From the inconspicuous viral fever syndrome to the fatal dengue shock syndrome/dengue haemorrhagic fever. There is a need for a simple predictive marker for patients at risk of progressing.

Various metabolic abnormalities have been found in dengue patients, namely, elevated liver enzymes, low sodium and ionised calcium levels, hyponatremia, increased urinary protein levels.

Proteinuria has been used as a method for assessing severity of dengue .proteinuria is best measured by 24 hrs urinary protein but it is a cumbersome procedure. Various texts

show that urine spot PCR as an acceptable alternative. Normal urine spot PCR is less than 30mg/mmol.

WHO 2009 guidelines classify dengue fever into dengue fever, dengue fever with warning signs and severe dengue fever. Dengue fever is patients with fever, rash, vomiting, aches and leucopoenia.

The warning signs in dengue are

• Abdominal pain or tenderness

• Persistent vomiting

• Clinical fluid accumulation

• Mucosal bleed

• Lethargy, restlessness

• Liver enlargement >2 cm

• Laboratory: increase in HCT concurrent with rapid decrease in platelet count

Severe dengue is defined as those with

Severe plasma leakage leading to:

• Shock (DSS)

• Fluid accumulation with respiratory distress

Severe bleeding as evaluated by clinician

Severe organ involvement

• Liver: AST or ALT >=1000

• CNS: Impaired consciousness

• Heart and other organs.

WHO guidelines 1997 defines dengue hemorrhagic fever as those with

- positive tourniquet test, petechiae, ecchymoses or purpura, bleeding from injection sites or other locations, malena or hematemesis,
- thrombocytopenia <1,00,000/mm3
- Plasma leakage as evidenced by increase in hematocrit 20% from baseline, decrease in hematocrit >20% after volume replacement, pleural effusion, ascites and hypoproteinemia.

Dengue shock syndrome is defined by Patients with dengue hemorrhagic fever with tachycardia. Pulse pressure <20mmhg, hypotension for age, cold skin, restlessness.

A laboratory criterion for confirmation is usually done by detection of IgM antibody.

Vasanwala et al showed a positive correlation with dengue hemorrhagic fever and dengue shock syndrome.

OBJECTIVE OF THE STUDY:

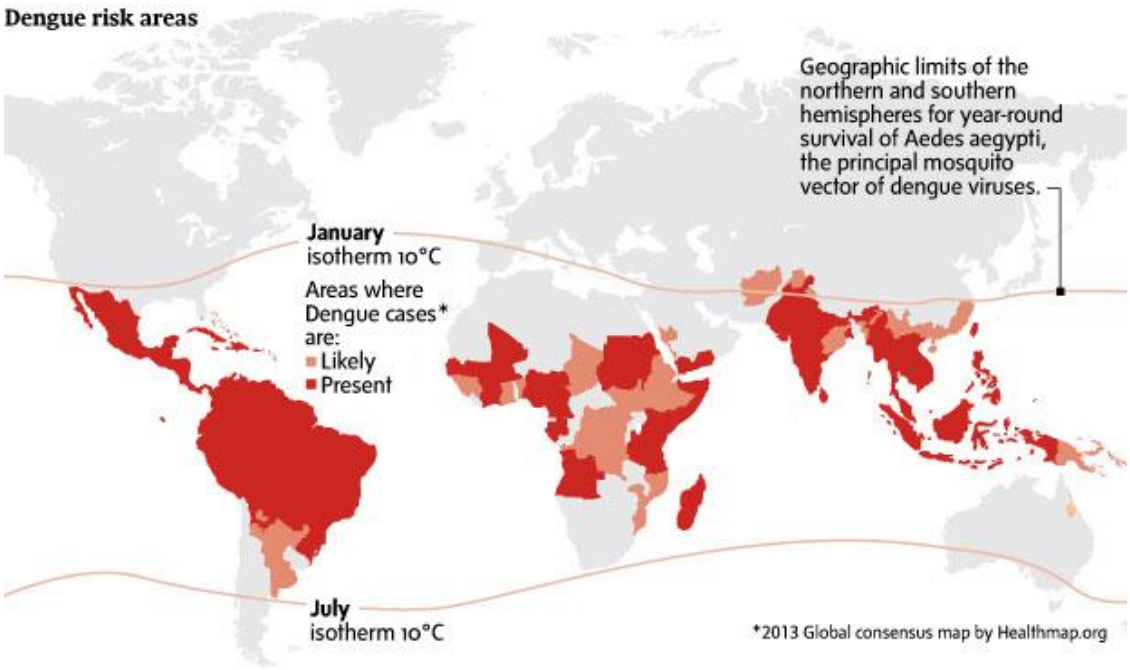
- To study daily urine spot protein creatinine Ratio in dengue fever patients
- To use it to predict patients at risk for developing complications
- To calculate its sensitivity and specificity as a prognostic tool

REVIEW OF LITERATURE

Dengue infections are caused by the four distinct dengue virus serotypes (DENV1, DENV2, DENV3, DENV4) of the Flaviviridae family of arboviruses are the most important vector borne diseases in humans, in geographical distribution, morbidity and mortality. The global burden of dengue has increased at least thirty times over the last 3 decades and there are now 2.5 billion people at risk of the disease. Annual dengue infections estimate stands at 50 million for symptomatic infections and about four hundred million for asymptomatic infection. Among these about 500000 severe cases of dengue hemorrhagic fever/dengue shock syndrome and 20,000 deaths occur with most of them being children and adolescents. The most commom vector is Aedes.aegypti. Clinical spectrum of dengue can range from, from a mild febrile illness known as ‘dengue fever’ through to ‘severe dengue’, previously known as dengue haemorrhagic fever (DHF), which is characterized by capillary leakage leading to hypovolaemic shock, organ impairment and bleeding complications. There are currently no

antiviral drugs and and the management relies on judicious fluid replacement of the severe cases. A vaccine dengue vaccia is under trial. The efficacy of which would be established in the near future.

Epidemiology

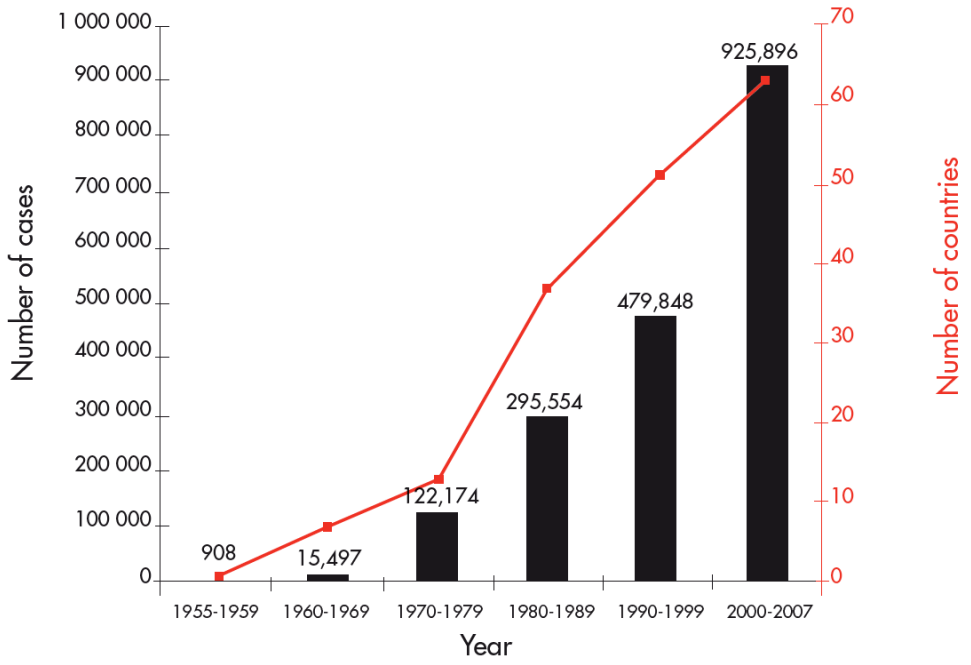


Dengue is occurs between 30°N and 40°S, where environment is optimal for *Aedes* mosquitoes to transmit the dengue virus.

Like its sibling the yellow fever it is likely that dengue virus was also transmitted by the slave trade and world wars across various countries. The first recorded outbreak of dengue occurred in Pennsylvania in USA.A similar outbreak was alaso reported in spain during the same time. The victims suffered severe pain which led to the term Break bone fever. Next occurred in the Australian mining towns.

After the world wars trade flourished between nations and travel became rampant leading to spread to new frontiers for the dengue virus. During the 1950s newer forms of the disease began to appear. The Thai hemorrhagic fever (Dengue hemorrhagic fever) which also occurred in surrounding nations. The trend continues and now over 70% of the global dengue cases occurs in Asia.

Figure 1.2 Average annual number of dengue fever (DF) and dengue haemorrhagic fever (DHF) cases reported to WHO, and of countries reporting dengue, 1955–2007



Dengue transmission occurs throughout the year in endemic tropical areas; however, in most countries there is a distinct seasonal pattern, with increased transmission usually associated with the rainy season. Outbreaks occur most frequently in areas where multiple serotypes of dengue virus are simultaneously endemic or sequentially epidemic and infections with heterologous types are frequent. In endemic areas dengue occurs most frequently in children aged between 2 and 15 years. Severe dengue is usually associated with secondary dengue infection and during primary infection in infants less than 1 year, born to dengue-immune mothers. It is clear that dengue

and other arboviruses with similar ecology had a widespread distribution in the tropics as long as 200 years ago.

DENGUE VIRUS:

The dengue virus is a member of *Flavivirus* genus in the family Flaviviridae. It is a single-stranded enveloped RNA virus, 30 nm in diameter. There are 4 closely related but distinct serotypes (DENV1–4). They have antigens that cross-react with other members of the same genus namely, yellow fever, Japanese encephalitis and West Nile viruses.

All 4 serotypes of DENV evolved independently from ancestral sylvatic viruses and have become both ecologically and evolutionarily distinct. The evolution of dengue virus is differerent in many important aspects from other flaviviruses, though they share many of the clinical features such as production of severe fever, headache, myalgia, hepatitis, encephalitis and haemorrhage. according to Vasilakis (Vasilakis et al., 2010) more detailed phylogenetic studies suggest an Asian origin for the dengue virus, where sylvatic cycles between

non-human primates and *Aedes*mosquitoes arose. Subsequent evolutions resulted in four antigenically and phylogenetically distinct serotypes: DENV-1, DENV-2, DENV-3 and DENV-4. Each of these four serotypes developed independently into an endemic cycle of transmission between humans and *Aedes albopictus* . This endemic cycle is separate from the sylvatic cycle. So, urban cycles of DENV can no longer be considered zoonotic.

The dengue evolves at a constant rate and the past strains have been in circulation with humanity for the past century. Genetic variations are also observed in between dengue viruses even from same countries, a proof of their diversity. The past three decades have seen a spurt in infections with DENV-2 and DENV-3. There are five genotypes identified in DENV-1; five in DENV-2; four in DENV-3; and four in DENV-4. Some of these subtypes differ in virulence and ability to cause disease.

Transmission

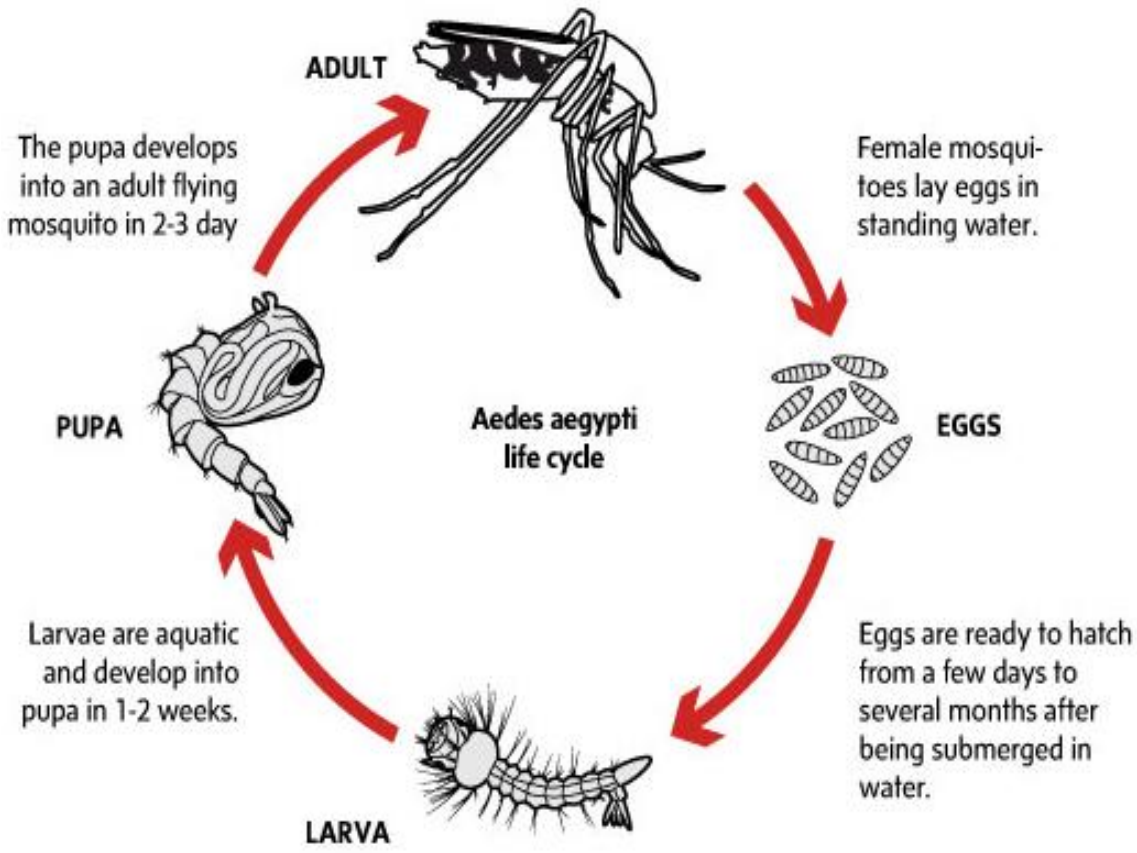
Dengue virus is transmitted from human to human by different species of *Aedes* mosquitoes. DENV circulation occurs in two cycles:

1) endemic/epidemic cycle between humans and peridomestic mosquitoes, *Aedes aegypti* and *Ae. albopictus*

2) a sylvatic enzootic cycle between non-human primates and several arboreal *Aedes* species.

Aedes aegypti is the most efficient of the mosquito vectors because of its domestic habits. The female mosquito feeds on humans during the day. After feeding on an infected person whose blood contains the virus, the female *Ae. aegypti* can transmit dengue, either immediately by a change of host when its feeding is interrupted or after an incubation period of 8–10 days, during which time, the virus multiplies in the salivary glands. Once infected, the mosquito host remains infective for life (30–45 days). *Ae. albopictus*, *Ae. polynesiensis* and several species of the *Ae. Scutellaris* complex are other aedes species

capable of transmission. Each of the species have their own particular geographical distribution and they are in general less efficient vectors than *Ae. aegypti*. Transovarian transmission of dengue viruses has been documented. Their epidemiological importance has not yet been established.



Pathogenesis

The strong association between the development of severe disease in secondary dengue and the observation that complications occur when the viraemia is in steep decline, has led to the suggestion that the pathogenesis of severe dengue is immune mediated. Halstead in the 1970s proposed the ‘antibody dependent immune enhancement theory’ (ADE) based on in vitro and primate studies. This association of sequential dengue infections being a risk factor for severity has been confirmed repeatedly in epidemiological studies, from different parts of the world. In addition, a particular sequence of infecting serotypes have been linked to severe disease, with several studies suggesting severe dengue is more common in a secondary infection with DENV2. During the second infection with a different dengue serotype, pre-existing antibody from the first infection fails to neutralize and may instead enhance viral uptake and replication in mononuclear cells. The resulting higher viral load has been linked to disease severity. Other factors that may contribute to the pathogenesis of severe dengue

include more virulent strains of the virus, host genetic factors, age and comorbidities.

HUMORAL IMMUNE RESPONSE

After an acute phase of infection by a particular dengue serotype, there is an antibody response to all four dengue serotypes. There is a long-lasting immunity to the homologous serotype of the infecting strain. A cross-reactive heterotypic immunity to all serotypes has been reported for period of 2–12 months following primary infection. The waning cross-reactive heterotypic antibody causes the occurrence of severe dengue through ‘antibody dependent enhancement’. The heterologous antibody acquired from a previous infection fails to neutralize the current infecting serotype, on the contrary it enhances the viral uptake into Fcy receptor-bearing cells, particularly monocytes and macrophages. ADE, in addition to facilitating viral entry into cells, also increases viral replication within the cells, through alterations of innate and adaptive intracellular antiviral mechanisms. Further research has proven that the antibodies in high titres fails to neutralize the virus. A different

example of ADE is in the setting of severe primary dengue in infants born to dengue-immune mothers. Maternally derived IgG antibody takes 4-12 months to disappear from the childs circulation. An infection by dengue virus may result in the catastrophic dengue hemorrhagic fever through the antibody mediated enhancement.

CELL-MEDIATED IMMUNITY

The cellular immune response is vital in controlling dengue infections, but newer studies have concluded that cell mediated immunity has a major role to play in the severity of dengue. The severity has been attributed to the increased T cell activity which provides no use in controlling the virus. Activated and infected monocytes and endothelia release with their lysis .TNF-α, IL-1, platelet-activating factor (PAF), IL-8, and RANTES, act in synergy with lymphokines, histamine, and virus and immune complex–induced C3a and C5a cause the temporary vascular endothelial dysfunction that leads to plasma leakage. While activated cells responding to cross-reactive antigens predominate over primary responses to the infecting

virus, following the paradigm of original antigenic sin, they are marked for apoptosis and are ineffective in viral clearance, and they may be a source of cytokines with a negative clinical effect. A study investigating T-cell responses to the entire dengue proteome, showed that the response was most marked to the non-structural protein 3 (NS3), with high cytokine and low CD107a (a marker of cell degranulation) predominating. This suggests that in severe dengue the low cytotoxic potential of the T cells fails to obtain early viral control, instead high cytokine-producing cells dominate the response with the excessive pro-inflammatory cytokines causing the tissue damage and plasma leakage. Other studies have linked disease severity to cellular markers of activation in plasma including interleukin-6 and soluble IL-2 receptor.

COMPLEMENT

Complement activation has been suggested to play a role in the pathogenesis of dengue. Studies have shown cross-reactive antibodies to the dengue virus can activate complement at the surface of endothelial cells. The release of C3a and C5a anaphylatoxins has been associated temporally with the onset of plasma leakage and shock. High levels of the major non-structural protein NS1 has been linked to disease severity. A study in Thailand demonstrated that NS1 was able to activate complements, directly on the endothelial cells infected by dengue virus, leading to local and systemic generation of anaphylatoxin C5a and the terminal SC5b-9 complexes. The plasma levels of NS1 and SC5b-9 complexes correlated positively with disease severity and these complexes were detected in pleural fluid from the patients with severe dengue. In addition, NS1 may evade immune system by modulating the classical and lectin complement pathways through reduced functional capacity of C4. These studies suggest a possible role for complement in the pathogenesis of severe dengue, both

through excessive local activation at endothelial surfaces contributing to vascular leakage as well as immune modulation leading to a higher viraemia. Recent animal studies tend to suggest that complements may also have a protective role. The C1q and Mannose binding lectin may play a crucial role inactivating DENV2. And patients with reduced MBL were shown to have greater severity.

HISTOPATHOLOGY:

From animal studies, after inoculation the dengue virus reaches the regional lymph nodes through the dendritic cells and disseminates to the reticuloendothelial system like spleen and also other organs in which it multiplies and from which it enters the blood causing the secondary viremia. Skin lesions in uncomplicated dengue fever seen in human were studied by biopsy. The chief pathology occurred around the small blood vessels and consisted of endothelial swelling, perivascular oedema and mononuclear cell infiltration. Extensive extravasation of blood without inflammatory changes were

observed in the petechial lesions. Significant changes found in major organ systems are

• vasodilatation, congestion, perivascular haemorrhage and edema of arterial walls

• Proliferation of RE cells with increased phagocytic activity were observed

• The lymphoid tissues showed increased activity of the B lymphocytes with proliferation of plasma cells and lymphoblastoid cells

• In the liver shows focal necrosis of the hepatocytes and Kupffer cells, with formation of Councilman-like bodies

• Dengue virus antigen is found predominantly in cells of the spleen, thymus and lymph nodes, in Kupffer cells and in the sinusoidal lining cells of liver and alveolar lining cells of the lung.

PATHOPHYSIOLOGY:

The hallmarks of severe dengue are plasma leakage and abnormal haemostasis. Clinical evidence supporting plasma leakage includes a rapid rise in haematocrit, hypoproteinaemia, pleural effusions and ascites and reduced plasma volume, leading to haemodynamic compromise and hypovolaemic shock. Microvascular leakage has been demonstrated using the non-invasive technique of strain gauge plethysmography. A study showed that the filtration capacity was higher in DHF patients than controls, but did not show a difference between the severity grades of DHF. They also showed that age-related changes occur in microvascular permeability, with children having higher filtration capacity than adults, which would explain why dengue shock syndrome is more common in childhood. The transient nature of the leakage implies a functional increase in vascular permeability. The microvascular leak occurs when the viral load is reducing and indicating that immune response is responsible for the detrimental effects. Disruption in the endothelial glycocalyx layer has been implicated, through

immune-mediated mechanisms by the virus or the NS1 antigen adhering to the endothelial layer. The NS1 antigen is a glycoprotein secreted from dengue-infected cells and is required for viral replication. Studies have shown that NS1 can selectively bind to heparan sulphate in the glycocalyx layer of microvascular endothelial cells. Thus facilitating immune complex formation and antibody-dependent complement activation causing the endothelial damage and microvascular leakage. The vascular leakage and hypoproteinaemia seen in the blood at defervescence is also associated with proteinuria. The proteinuria tends to occur more frequently in severe dengue and urinary protein/creatinine ratio has been suggested as a predictor of progression to severe disease. A study in Vietnamese children demonstrated a reduction in different size proteins in plasma was associated with increased fractional urinary clearance of the same proteins. The proteinuria resolves in the convalescent period and there is generally no associated renal damage with no increase in creatinine noted. A transient glomerulonephritis has

been suggested from renal biopsies, with deposition of dengue immune complexes in the glomerular basement membrane.

The abnormalities in haemostasis and hemotology seen in dengue infections include the following:

(1) vasculopathy;

(2) thrombopathy, due impaired platelet functioning and production leading to moderate to severe thrombocytopenia

(3) coagulopathy, due to activation of the coagulation system and fibrinolysis, later stages of severe disease causing disseminated intravascular coagulation (DIC)

(4) bone marrow changes, include depression of all elements of marrow , with maturation arrest of megakaryocytes even during the early phase of the illness, which is reversed after defervescence period.

The most consistent finding in dengue infections is a transient thrombocytopenia. The exact underlying mechanism still remains unclear. Studies suggest that it may be multifactorial, including:

- suppression of megakaryocytopoiesis
- increased platelet clearance by DENV-induced apoptosis
- antiplatelet antibodies.

Activation of the coagulation system and fibrinolysis has been demonstrated in various studies and is more pronounced in severe cases. One study showed that persistently elevated levels of tissue activator inhibitor levels and an increased thrombin-antithrombin to plasmin-antiplasmin ratio was associated with poorer outcomes in dengue patients. Other studies have shown that mild prolongation of prothrombin time and partial thromboplastin time and reduced fibrinogen are also associated with poorer outcomes. Fibrinogen degradation products are rarely raised, however not to levels consistent with a classical DIC picture. Theories to explain these coagulation abnormalities' include; through capillary leakage various coagulation factors are lost and leading to the coagulation abnormalities. The prolongation of the PT and APTT also coincides with maximal vascular leakage and is more

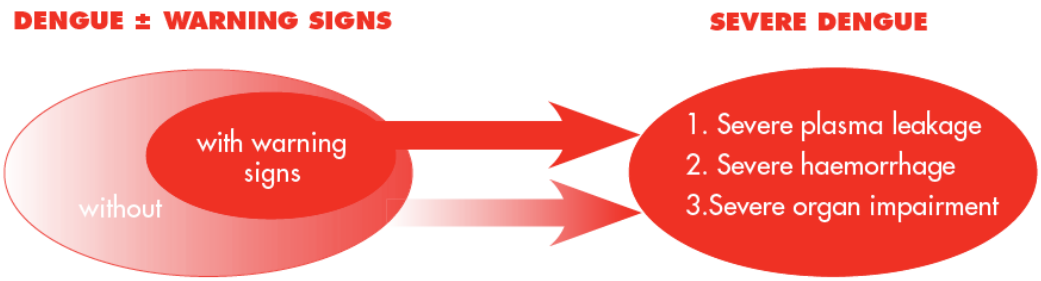
pronounced in these severe patients. In vitro studies have shown that the virus can bind and activate plasminogen and moreover cross-reactive antibodies to plasminogen have been demonstrated in acute and convalescent serum of dengue patients.

CLINICAL FEATURES:

Dengue fever presents with a wide spectrum of clinical features, from a simple febrile illness to severe features of plasma leakage like dengue hemorrhagic fever or Dengue shock syndrome leading to life-threatening consequences. Dengue was previously classified into dengue fever and dengue haemorrhagic fever (DHF) which had four grades, with DHF III and IV together called as dengue shock syndrome (DSS). In 2009, the WHO reclassified dengue due to difficulty arising in applying the old classification system in clinical situations and more reports of severe cases not fitting the criteria for DHF.

The new classification emphasizes severity with patients being classified into

1. dengue with or without warning signs
2. severe dengue



CRITERIA FOR DENGUE ± WARNING SIGNS

Probable dengue

live in /travel to dengue endemic area.
Fever and 2 of the following criteria:

- Nausea, vomiting
- Rash
- Aches and pains
- Tourniquet test positive
- Leukopenia
- Any warning sign

Laboratory-confirmed dengue

(important when no sign of plasma leakage)

Warning signs*

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargment >2 cm
- Laboratory: increase in HCT concurrent with rapid decrease in platelet count

*(requiring strict observation and medical intervention)

CRITERIA FOR SEVERE DENGUE

Severe plasma leakage

leading to:

- Shock (DSS)
- Fluid accumulation with respiratory distress

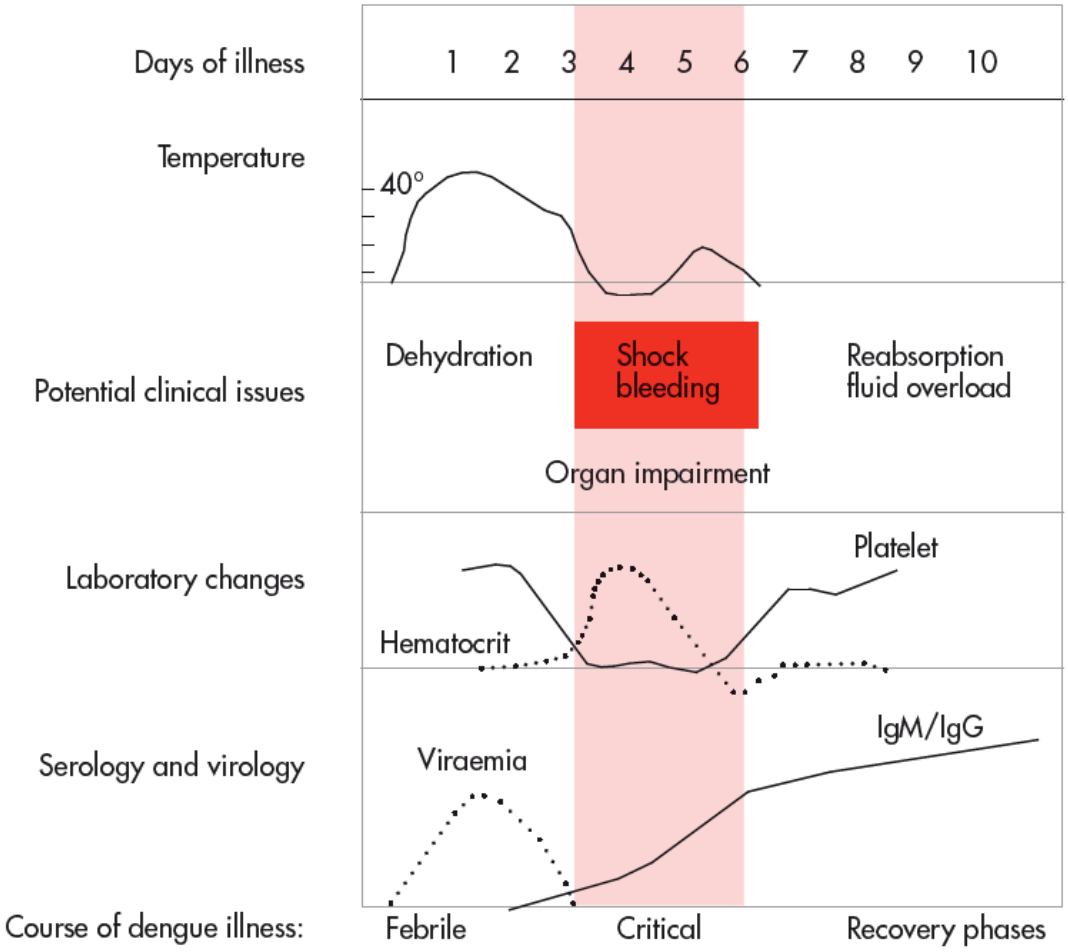
Severe bleeding

as evaluated by clinician

Severe organ involvement

- Liver: AST or ALT >=1000
- CNS: Impaired consciousness
- Heart and other organs

Specific organ impairment can also occur without shock or any other features of severe dengue, including hepatitis, encephalitis and myocarditis. Although a mild increases in hepatic transaminases are usually present, rarely ALT levels >1000 are seen with associated liver dysfunction. Dengue fever can also present as an acute encephalitis syndrome without other manifestations of the disease is increasingly seen in endemic areas. It is more common in children and although rarely fatal a handful are left with neurological sequelae. Different cardiac manifestations have been reported in dengue patients like acute myocarditis, conduction abnormalities and myocardial depression.These patients need to be identified early in order to tailor their fluid management. The clinical course consists of febrile, critical and recovery phases.



FEBRILE PHASE

After an usual incubation period of 5–8 days following an infected mosquito bite, the disease begins with a sudden onset of fever along with severe headache and chills, pain behind the eyes, particularly on eye movement, backache and pain in the muscles, bones and joints. These symptoms during the Spanish epidemic made it gain the name break bone fever. During the

febrile period the temperature can increase high as 40°C and the fever may be sustained for 5–6 days and can occasionally have a biphasic course. Anorexia, vomiting and abdominal pain are also common. As the disease progresses the patient becomes toxic and may show marked weakness and prostration. Other reported symptoms include sore throat, altered taste sensation, constipation and depression.

Several types of skin rash have been described. Initially, diffuse flushing, mottling or fleeting pinpoint eruptions may be observed on the face, neck and chest. These are transient in nature and occur on the 2nd day.

A second type of skin rash is a conspicuous rash that may be maculopapular or scarlatiniform and appears on approximately the 3rd or 4th day when fever starts to subside. This rash starts on the chest and trunk and spreads towards the extremities and face and may be accompanied by itching and skin hyperaesthesia. Towards the end of the febrile period or immediately after defervescence the generalized rash fades and localized clusters of petechiae may appear over the dorsum of the feet and on the legs, hands and arms. This confluent petechial rash is characterized by a scattered pale round area of normal skin. Mild haemorrhagic complications occur during the febrile phase, presents as scattered petechiae on extremities, axillae, trunk and face. A positive tourniquet test and/or tendency to bruise at venepuncture sites are usually seen.

Bleeding into the skin, from the nose, gums and gastrointestinal tract are common but haematuria is rare. The liver can often be enlarged, but jaundice is usually not seen except for liver enzyme elevation. A normal white blood count or leucopenia is common and neutrophils may predominate initially. Towards the end of the febrile phase there is a reduction in the number of total leucocytes and neutrophils shortly before or simultaneously with a relative increase in lymphocytes with the presence of atypical lymphocytes. The

leucopenia usually reaches a nadir shortly before the temperature and platelets drop. This observation is valuable in marking the end of the febrile period and the beginning of the critical phase. Other changes include hypoproteinemia, hypoalbuminemia, hyponatremia and mildly elevated alanine aminotransferase (ALT)/aspartate aminotransferase levels (AST).

CRITICAL PHASE

It is not possible to predict who will have an uneventful defervescence and who will go on to develop severe dengue. However, using the WHO warning signs with it may be possible to expect complications the patients.

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy/restlessness
- Liver enlargement >2 cm
- Laboratory increase in HCT concurrent with rapid decrease in platelet count

Particular vigilance during the critical phase can help to identify which patients will require more intensive supportive

therapies. The critical phase, which usually occurs at fever defervescence around day 5, is the period where an increase in the capillary permeability and plasma leakage can occur. This may present clinically as pleural effusion and/or ascites depending on the degree of plasma leakage and once the critical volume is lost, circulatory failure occurs.

The skin becomes cold and clammy and the pulse pressure becomes narrow (20 mmHg) with elevation of diastolic pressure to meet the systolic pressure. This narrowing of pulse pressure is the earliest marker in BP signalling impending circulatory failure.

The platelet count drops shortly before or simultaneously along with the haematocrit rise (20%) and both changes occur before the subsidence of fever and before onset of shock. Clotting abnormalities are usually present including prolongation of prothrombin and partial thromboplastin time and reduced fibrinogen, which has been shown to correlate to severity of disease but not bleeding. Without appropriate fluid management the patient can deteriorate into profound shock

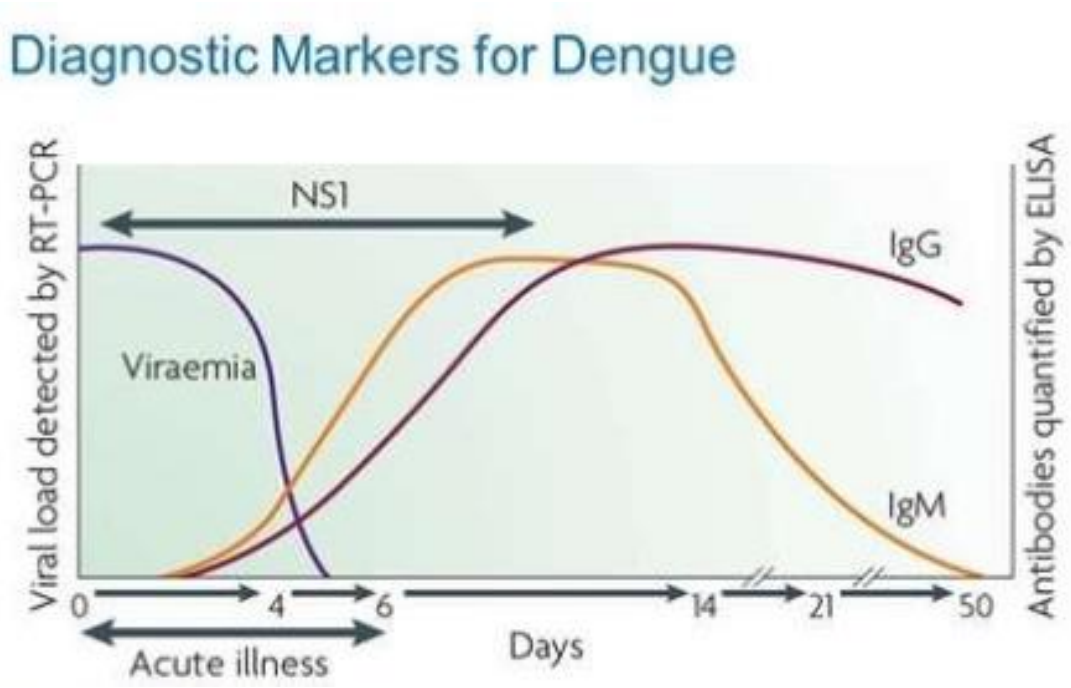
with an imperceptible pulse and blood pressure. Prolonged shock is often complicated by metabolic acidosis, multi-organ impairment and severe bleeding which carries a poor prognosis. The critical phase usually lasts for 24–48 hours, during which time the clinically significant plasma leakage can occur, after which time the recovery period begins.

RECOVERY PHASE

The extra vascular fluid begins to be reabsorbed over the next 48–72 hours. If intravenous fluids are continued into this period there is significant risk of fluid overload, manifesting as respiratory distress from pleural effusions and/or ascites. General symptomatic improvement is seen, with return of appetite, haemodynamic stability and diuresis. During this period, the white cell count begins to rise followed by the platelets, the haematocrit may drop in part due to the dilutional effects of the reabsorbed extravascular fluid.

LABORATORY DIAGNOSIS:

Diagnosis can be confirmed by serological testing and virus detection by molecular techniques or less frequently by virus isolation. No single diagnostic test performed in isolation is sufficiently sensitive to diagnose all the different stages of dengue infection. In the first 3–5 days of the infection during the febrile phase, RT-PCR techniques to detect DENV RNA in the blood are the most sensitive and specific test, however after defervescence, this method becomes less useful as the viraemia falls.



Serological diagnosis by detection of anti-dengue IgM and IgG by enzyme-linked immunosorbent assay (ELISA) can be used to distinguish primary and secondary infection, but lacks sensitivity in the early stages of the disease. IgG serology lacks sensitivity in early stages of the disease and requires paired serum samples, it also lacks specificity due to cross reactions with other flaviviruses. IgM antibody capture (MAC) – ELISA is specific in distinguishing dengue from other flavivirus infections and has the advantage over the haemagglutination test in that a definite diagnosis can be made from an acute blood specimen alone, with a sensitivity of about 78%; when convalescent sera are tested the sensitivity is 97%.

IgG antibodies to dengue virus antigens increase rapidly in patients with secondary dengue infection. A diagnostic (fourfold) increase in dengue antibody by the haemagglutination inhibition test can be demonstrated from a paired sera obtained early in the febrile phase or on admission and 3–5 days later. A third specimen 2–3 weeks after onset is, however, required to confirm diagnosis of primary dengue infection.

More recently an ELISA assay for dengue non-structural protein 1 (NS1) detection has been developed and commercial test kits are now available. They are useful additions to diagnose dengue with excellent sensitivity and specificity in early disease, with limitations later on in the disease and in those with a concurrent humoral immune response.

TREATMENT:

The treatment of dengue involves various aspects of the disease. During early phase focus on the temperature reduction, during the defervesence phase focus on the optimal fluid management and careful watch for any bleeding signs and transfusion if needed. During resolution phase focussing in the problems with volume overload when the extravasated fluid reenters the blood stream.

EARLY FEBRILE PHASE:

Patients should be adviced to take adequate hydration and plenty of juice. Oral rehydration solutions may also be prescribed. Fever can be controlled by tepid sponging, use of

paracetomol. The use of NSAIDS like aspirin, ibuprofen are to be avoided as they can irritate the gastrointestinal tract and aggravate vomiting and also increase the risk of gastrointestinal bleed. They also inhibit platelet functions which may compound he risks of thrombocytopenia in the defervesence phase.

CRITICAL PHASE:

Critical phase mainly involves the management of fluid. The various factors discussed above contribute to endothelial damage, vasodilation and increase in vascular permeability leading to loss of intravascular volume. During the phase it is essential to categorize patients to groups A-C. Group A patients may be advised oral fluids as much as they like with a lower limit of altleast 2-3L. These patients are the patients who don’t have any warning signs and ambulatory.

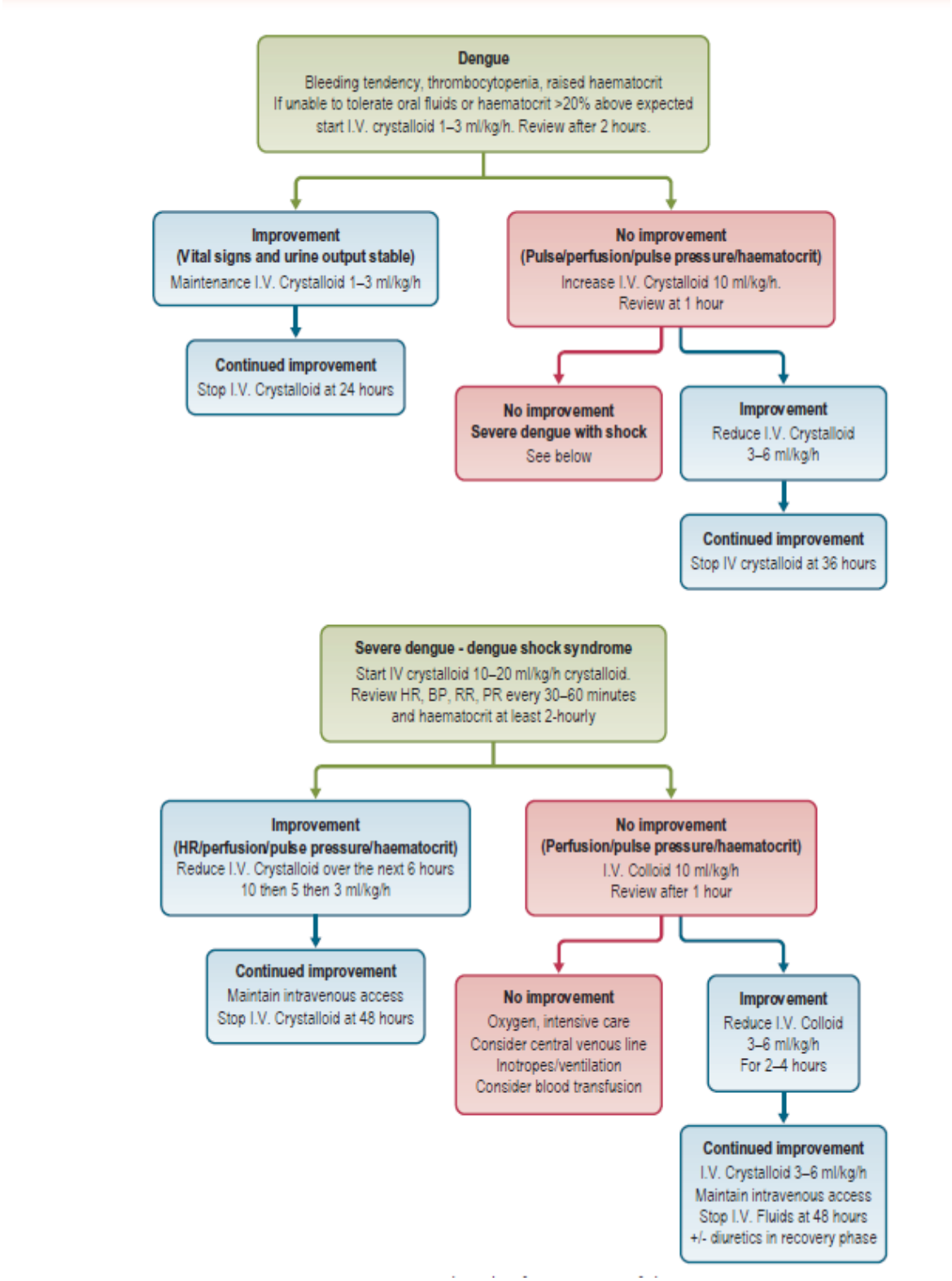
The next group B with warning signs but stable haematological status are given IV maintenance fluids mostly ringer lactate solution at 2-3ml/Kg/hr. These patients are to monitored closely for any sign of deterioration to severe dengue.

Urine output, vitals and fluid intake are to monitored every 6 hrs.

Group C are the patients who have severe dengue. It is appropriate to treat these patients in a tertiary care center with facilities for blood product transfusions.

Parameters	Stable circulation	Compensated shock	Hypotensive shock
Hypotensive shock	Clear and lucid	Clear and lucid (shock can be missed if you do not touch the patient)	Change of mental state (restless, combative)
Capillary refill time	Brisk (<2 sec)	Prolonged (>2 sec)	Very prolonged, mottled skin
Extremities	Warm and pink extremities	Cool peripheries	Cold, clammy extremities
Peripheral pulse volume	Good volume	Weak and thready	Feeble or absent
Heart rate	Normal for age	Tachycardia	Severe tachycardia with bradycardia in late shock
Blood pressure	Normal for age Normal pulse pressure for age	Normal systolic pressure but rising diastolic pressure Narrowing pulse pressure Postural hypotension	Narrowed pulse pressure (<20 mmHg) Hypotension (see definition below) Unrecordable blood pressure
Respiratory rate	Normal for age	Tachypnoea	Metabolic acidosis hyperpnoea/ Kussmaul's breathing

In patients presenting with shock the following algorithim is used.



Patients at risk of major bleeding are those who have:

–prolonged/refractory shock

–hypotensive shock and renal or liver failure and/or severe and persistent metabolic acidosis;

–given non-steroidal anti-inflammatory agents;

–pre-existing peptic ulcer disease;

–on anticoagulant therapy;

–any form of trauma, including intramuscular injection.

An useful criteria of transfusion of platelets is

- counts less than 10,000
- bleeding manifestation of clinical significance at any platelet count

For patients with loss of blood it is better to replace with packed cell and clinical improvement occurs when adequate volume is replaced.

Any patients with fall in hematocrit of more than 20% from baseline is a good candidate w requiring transfusion of packed cell approximately 10-20ml/Kg bodyweight.

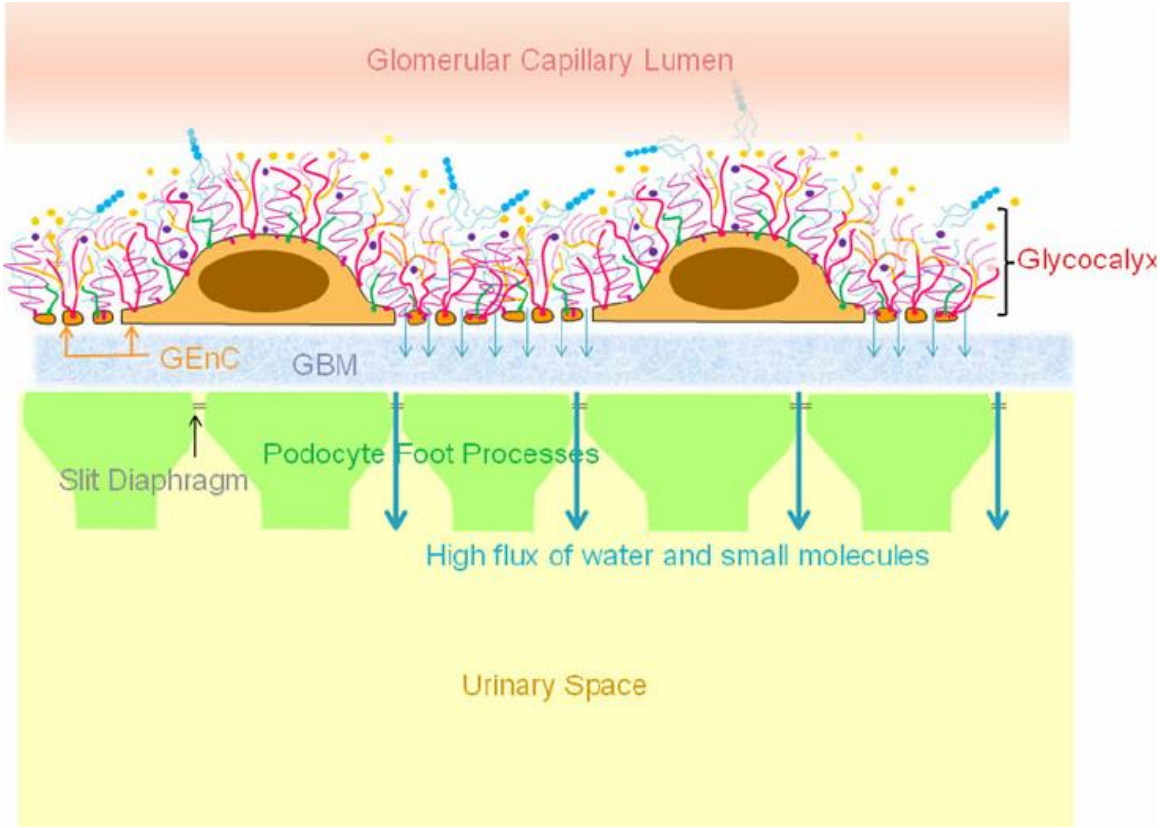
Preferentially fresh packed cells are to be transfused as stored ones have a low 2-3DPG which increases the oxygen affinity. Fresh packed cells have low 2-3 DPG which is optimal for the tissue perfusion.

DIFFERENTIAL DIAGNOSIS:

Conditions that mimic the febrile phase of dengue infection	
Flu-like syndromes	Influenza, measles, Chikungunya, infectious mononucleosis , HIV seroconversion illness
Illnesses with a rash	Rubella, measles, scarlet fever, meningococcal infection, Chikungunya, drug reactions
Diarrhoeal diseases	Rotavirus, other enteric infections
Illnesses with neurological manifestations	Meningo/encephalitis Febrile seizures
Conditions that mimic the critical phase of dengue infection	
Infectious	Acute gastroenteritis, malaria, leptospirosis, typhoid, typhus, viral hepatitis, acute HIV seroconversion illness, bacterial sepsis, septic shock
Malignancies	Acute leukaemia and other malignancies
Other clinical pictures	Acute abdomen – acute appendicitis – acute cholecystitis – perforated viscus Diabetic ketoacidosis Lactic acidosis Leukopenia and thrombocytopaenia ± bleeding Platelet disorders Renal failure Respiratory distress (Kussmaul's breathing) Systemic Lupus Erythematosus

PROTEINURIA

In a normal kidney, large quantities of high-molecular-weight (HMW) proteins traverse the glomerular capillaries, mesangium, without entering the urine space. Capillary wall being charge and size selective prevents most of these protein from entering the tubules but for a very tiny fraction of albumin and other proteins.



Proteins smaller than <20,000 Daltons can pass readily across the capillary wall. The concentration of such proteins in the plasma is very low and hence their volume in the urine remains very low. These low molecular weight proteins are reabsorbed by the proximal tubule. So, proteins likes α2-microglobulin, apoproteins, enzymes, and other peptide hormones are excreted in only very small amounts in the urine. Most healthy persons excrete between 30 and 130 mg/day of protein. The upper normal limit of total protein excretion in urine is generally given as 150 to 200 mg/day for adults. The upper limit of normal albumin excretion in urine is usually 30 mg/day.

A very small amount of protein normally appears in the urine as a result of normal tubular secretion. Tamm- Horsfall protein is an High Molecular Weight glycoprotein which is formed on the epithelial surface of the thick ascending limb of the loop of Henle and early distal convoluted tubule. Tamm-Horsfall protein, also known as the *uromodulin,* binds and inactivates the cytokines IL-1 and tumor necrosis factor.

METHODS FOR MEASURING URINARY PROTEINS

1. DIPSTICK METHOD

This method relies on the principle that presence of protein in a buffer solution causes a change in the pH of the solution which is proportional to the concentration of the protein. The dipstick contains a pH indicator dye and a buffer. The indicator color changes ranging from pale green to green to blue when stick is dipped in urine containing the proteins .The test is more sensitive to albumin and less sensitive to immunoglobulin light chains and other globulins. The test is very sensitive enough to detect even 20mg/dl of protein in urine. But it doesnot take into account the volume of urine. When urine volume is high and the urine is very much dilute, a relatively large amount of protein can even go undetected. If a total protein excretion approaching approximately 1 g/day may not be detected if urine output is as high as 10L/day as the concentration of urine protein falls below 20mg/dl.

The dipstick reports are interpreted as follows

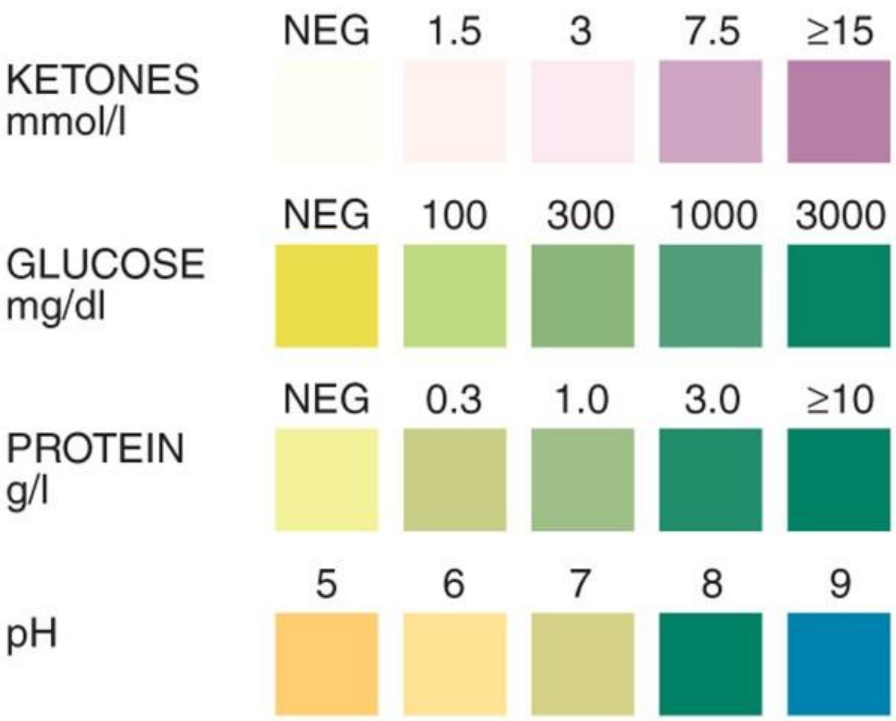
1. Negative <15mg %

2. 1+ 30-10mg/dl

3. 2+ 100-300mg/dl

4.3+ 300-1000mg/dl

5. 4+ >1000 mg/dl



Extremely alkaline(pH>7) urine produces a false positive result. Now albumin specific dipsticks are available and these are very useful to detect low grade albuminuria. Some strips can also measure albumin: creatinine ratio.

2. SULFOSALICYLIC ACID METHOD:

In this commonly used method, sulfosalicylic acid is added to a sample of urine, and the turbidity which occurs is measured with help of a photometer or nephelometer. Protein can be quantified through the comparison of the turbidity of the sample and that of a standard. This method lacks precision, and the variation is as high as 20%. A large number of proteins can be detected with this method, which includes γ-globulin light chains and albumin. This method is more sensitive to albumin than to globulins.

Trichloroacetic acid can also be used in place of sulfosalicylic acid which increases the sensitivity to γ-globulin. False-positive reactions may also occur due to high levels of

tolmetin sodium , tolbutamide, various antibiotics, and radio-contrast agents..

3.24 HOUR URINARY PROTEIN EXCRETION:

It gives average variation of protein excretion that occurs as a result of circadian rhythm and it is the most accurate method for monitoring proteinuria. This method detects albumins, globulins, and immunoglobulin light chains. This methods quantifies total protein rather than just albumin and hence his results in the detection of light chains.

4. PROTEIN CREATININE RATIO

The spot PCR is obtained by the ratio between urine protein excretion (measured by 24-Hour Protein Excretion or spot urine sample) and creatinine excretion, expressed as mg/mg or mg/mmol. Spot PCR represents a practical alternative to the 24-hour urine collection because it is easier to obtain and is not influenced by variations in water intake or diuresis. And the same sample can also be used for investigations under microscope.

A close correlation between the PCR in a random urine sample and the 24-hour protein excretion has been demonstrated in a wide range of patients, including those with different types of glomerulonephritis (GN) evaluated longitudinally during treatment. However, the results may be influenced by variations in creatinine excretion because it is dependent on the muscle mass. So in elderly and female patients, PCR values can be higher than the PCR in young men. The other factor to be taken in consideration is the timing of the sample, which can be influenced by the circadian fluctuation of protein excretion which in presence of minimal corresponding variation of creatinine excretion. So the best estimates are probably the ones obtained in the early morning.

Some consider that a normal PCR is sufficient to rule out pathologic proteinuria, but that an elevated PCR should be confirmed and quantified with a 24-hour collection. Advantages of spot PCR include

1. Not cumbersome
2. Not affected by diuretics or variation in water intake
3. The sample can be used for microbial analysis

5. SPECIFIC PROTEIN ASSAYS

Like electrophoresis, SDS –PAGE are newer more sensitive and accurate methods of protein estimation. The latter identifies different proteins in the urine by molecular weight and it is also useful to find out the pattern of proteinuria.

Immunoglobulin light chain excretion is suspected if the dipstick protein isnegative but 24 hour urinary protein is elevated. Immunofixation is used to confirm.

Grading of Spot PCR

Normal or mildly increased - <150mg/g

Moderately increased - 150-500mg/g

Severely increased - >500mg/g

PROTEINURIA AND DENGUE FEVER:

The relationship between dengue and proteinuria basically depends on the pathogenesis underlying all the complications of dengue. The dengue virus after the primary period of viremia get into macrophages and secondary viremia then ensues. The host immune system then develops antibodies against the dengue virus. These antibodies bind with the dengue virus and form a complex. The viremia has a large number NS1 antigens in the blood circulation. This NS1 alters the glycocalyx of in the glomerulus and thereby reducing the negative charge. This reduction in negative charge alone is not enough to produce the proteinuria. The deposition of antigen antibody complexes in the endothelium causes endothelial damage which also contributes to the proteinuria.

The dengue fever by various other mechanisms can also cause renal injury. Acute endocapillary Glomerulonephritis with IgG, IgM and C3 deposition presenting with hematuria, proteinuria and renal failure has been reported in dengue patients.

Kidney involvement in dengue includes AKI, proteinuria (sometimes nephrotic), GN, and HUS. Peak proteinuria has been suggested to potentially predict DHF in patients with dengue. The reported incidence of dengue-associated Acute Kidney Injury is extremely variable, from 1% to approximately 30%, but acute kidney injury development is a poor prognostic marker.

Dengue-induced AKI is usually associated with

1. shock
2. hemolysis,
3. Rhabdomyolysis
4. DHF grade IV and obesity in children
5. DSS in adults

MATERIALS AND METHODS

STUDY POPULATION:

SOURCE OF DATA:

The study will be conducted on 100 adult dengue fever patients admitted in medicine wards of Government Rajaji Hospital & Madurai Medical College during the study period from march 2018 to August 2018.

INCLUSION CRITERIA:

- Patients admitted for fever with thrombocytopenia
- IgM dengue ELISA positive
- Normal serum urea/creatinine at presentation

EXCLUSION CRITERIA:

- Pre existing Chronic Kidney Disease/nephrotic/nephritic syndrome
- Diabetes
- Hypertensives

- Coronary Artery Disease
- Female patients during menstrual cycle
- Urinary tract infections

ANTICIPATED OUTCOME:

- Urine spot protein creatinine Ratio will help in predicting patients at risk for progressing to severe dengue

DATA COLLECTION

Patients admitted with probable dengue will be explained the purpose & procedure of study , written informed consent in Tamil will be obtained. The selected patients will be evaluated as per pro forma. Only patients with confirmed dengue by IgM ELISA will be included.

Daily morning first void clean catch mid stream urine will be collected and sent for analysis. Daily morning complete blood counts are also collected.

LABORATORY INVESTIGATIONS

1. Complete blood count
2. Blood sugar
3. Serum urea
4. Serum creatinine
5. Daily morning first void mid stream urine spot protein creatinine Ratio
6. IgM ELISA for dengue
7. Ultra sonogram of abdomen

DESIGN OF STUDY:

Prospective study.

PERIOD OF STUDY:

6 MONTHS (MARCH 2018 to AUGUST 2018)

COLLABORATING DEPARTMENTS:

DEPARTMENT OF GENERAL MEDICINE

DEPARTMENT OF BIOCHEMISTRY

DEPARTMENT OF RADIOLOGY

ETHICAL CLEARANCE: OBTAINED

CONSENT: Individual written and informed consent

CONFLICT OF INTEREST: Nil

FINANCIAL SUPPORT: Self

DATA ANALYSIS:

Data entry was done in excel spread sheets and statistical analysis was done using sigmastat software. Chi square test was used in the analysis for p value between spot PCR and occurrence of DHF/DSS. The analysis of data was carried out by entering the coded information and generating tables. The data will be presented using descriptive statistics in form of tables and graphs. Results are expressed as proportions with 95% confidence interval. Univariate analysis was carried out using non –parametric Mann Whitney Test to compare the various parameters between two groups.

PROFORMA

Name:

Age / Sex: Residence: Rural / Urban

Occupation:

Total duration of illness :

Presenting complaints:

Past History:

H/o DM, HT, CKD, CVD, CAD, Thyroid disorders, Alcohol intake

Menstrual history:

Clinical Examination:

General Examination:

Consciousness,

Pallor,

Cyanosis

Jaundice,

Clubbing,

Lymphadenopathy,

Pedal oedema

Vitals:

PR

BP

RR

SpO2

Anthropometric measures:

Height (cm): Weight (kg): BMI:

Systemic examination:

CVS:

RS:

ABDOMEN:

CNS:

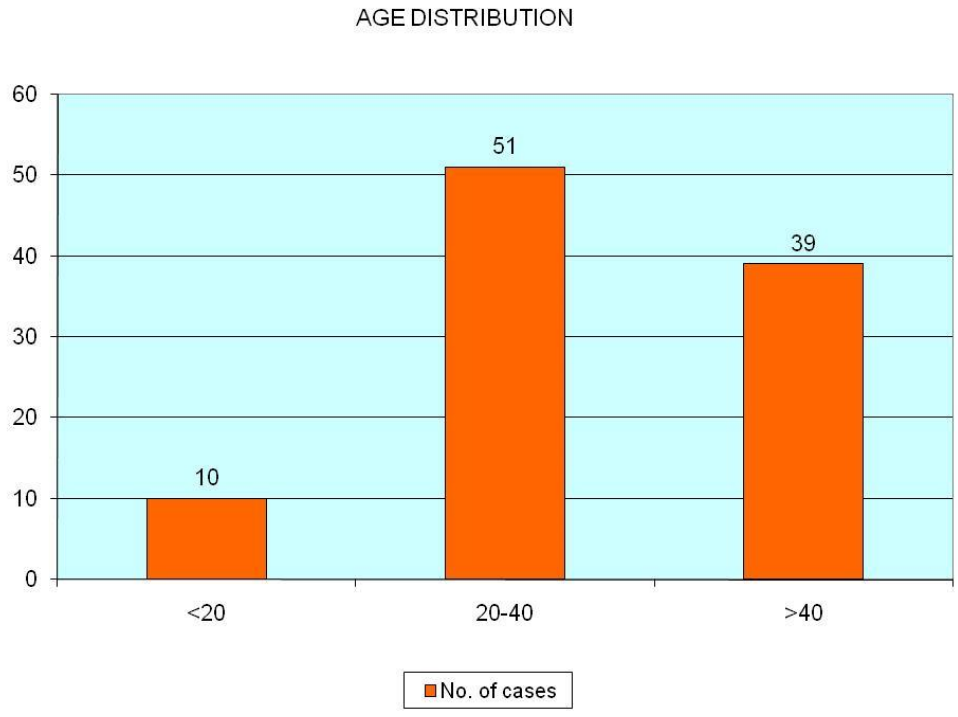
	Day -1	**Day 0***	**Day 1**	**Day 2**	**Day 3**	**Day 4**
Spot PCR						
Platelet count						
Warning signs						
Required transfusion						
hypotension						

*Day 0 is taken as the day of defervescence

RESULTS AND INTERPRETATION

Table 1: Age distribution among study population

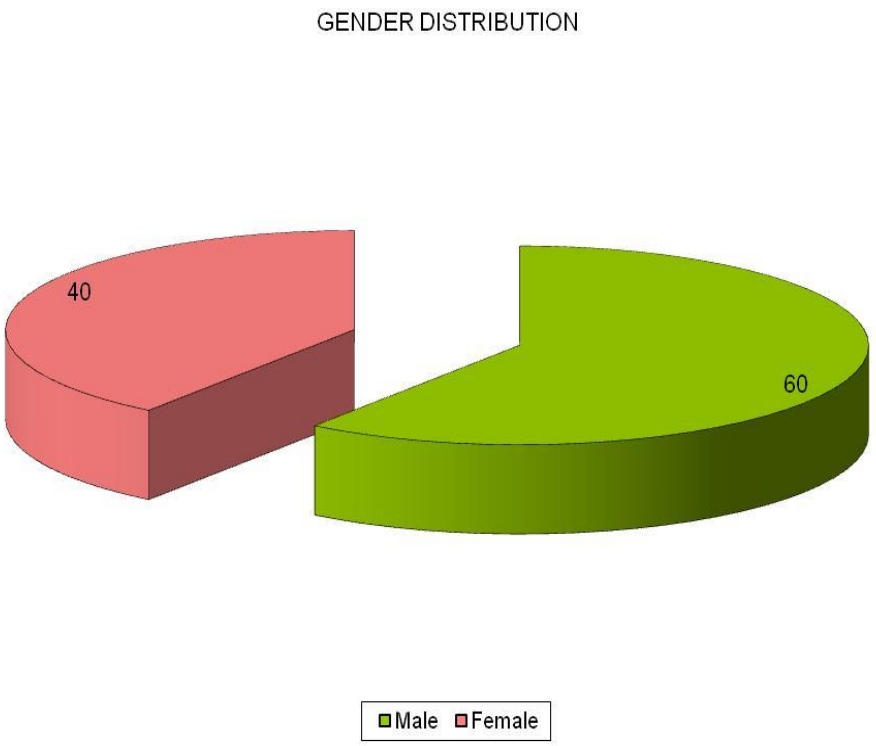
Age in years	No. of cases
<20	10
20-40	51
>40	39
Total	100



Of the total 100 patients, those of age less than 20 constituted were 10, between 20-40 yrs were 51 nad more than 40 were 39.

Table 2: Sex distribution among study population

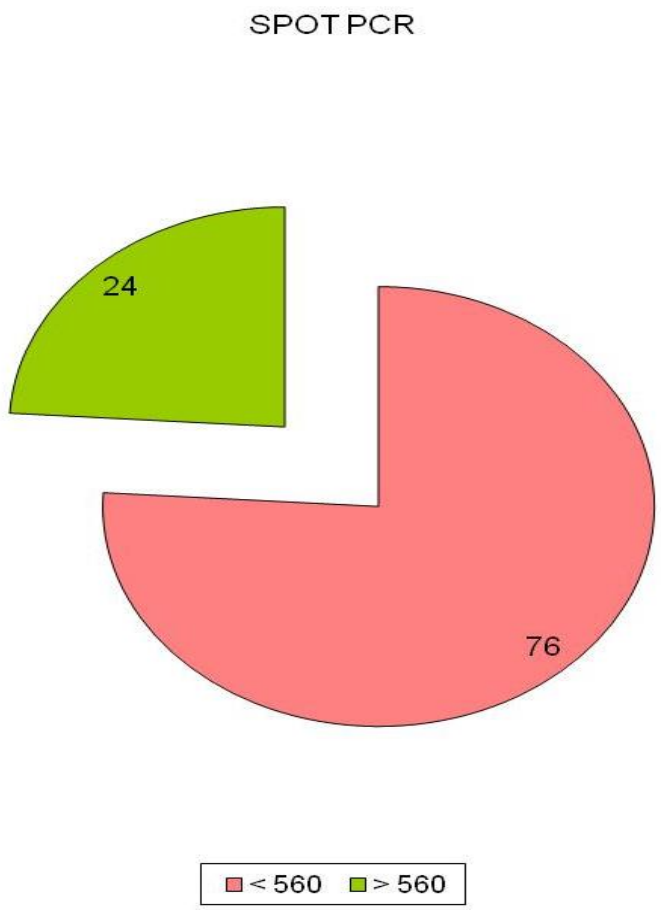
Sex	No. of cases
Male	60
Female	40
Total	100



Among the subjects the total number of men were more accounting 60 and female were 40.

Table 3: Distribution of urine Spot PCR among study population

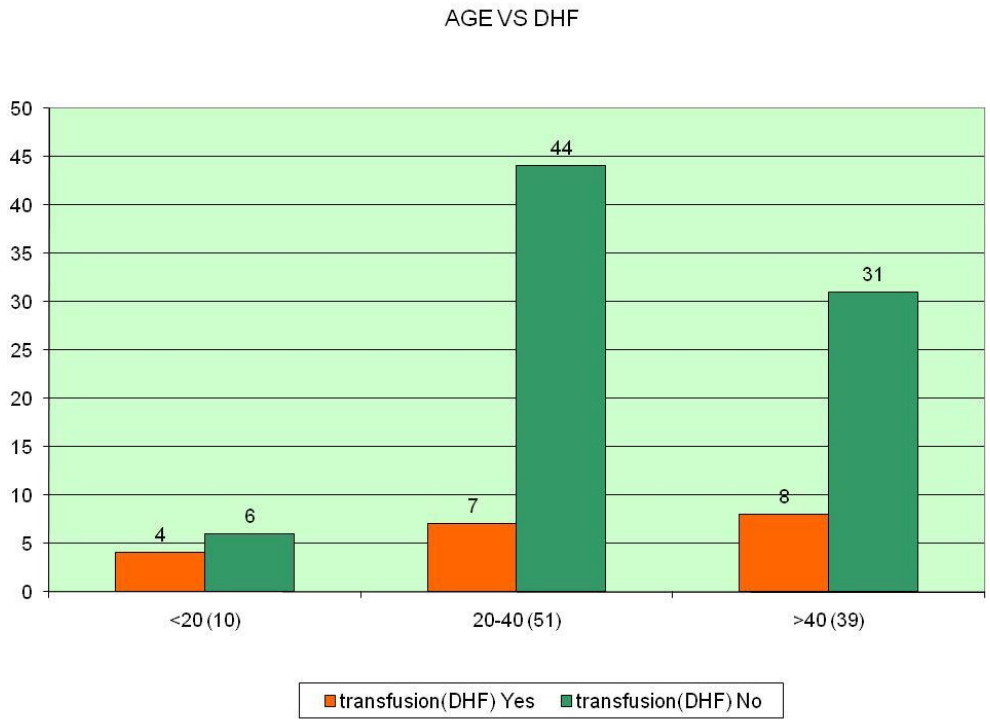
SPOT PCR	No. of cases
≤ 560 mg/g	76
> 560 mg/g	24
Total	100



Among the total 100 patients 24 had urine Spot PCR more than 560 mg/g and 76 had urine spot PCR less 560 mg/g.

Table 4: Age wise distribution among DHF patients

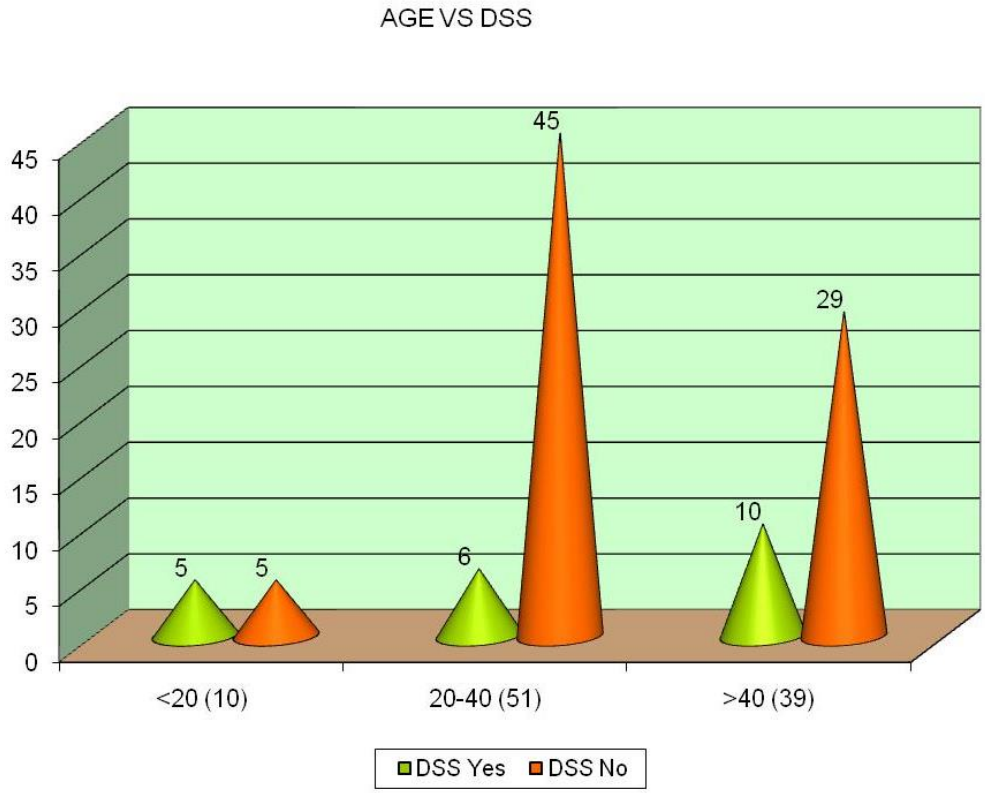
Age vs DHF	transfusion(DHF)	
	Yes	No
<20 (10)	4	6
20-40 (51)	7	44
>40 (39)	8	31
Total	19	81



Among the < 20 yrs age group there was 40 % incidence of DHF, among the 20-40 yrs group incidence was 13% and among the >40yrs group it was 20.5%.

Table 5:Age wise distribution among DSS patients

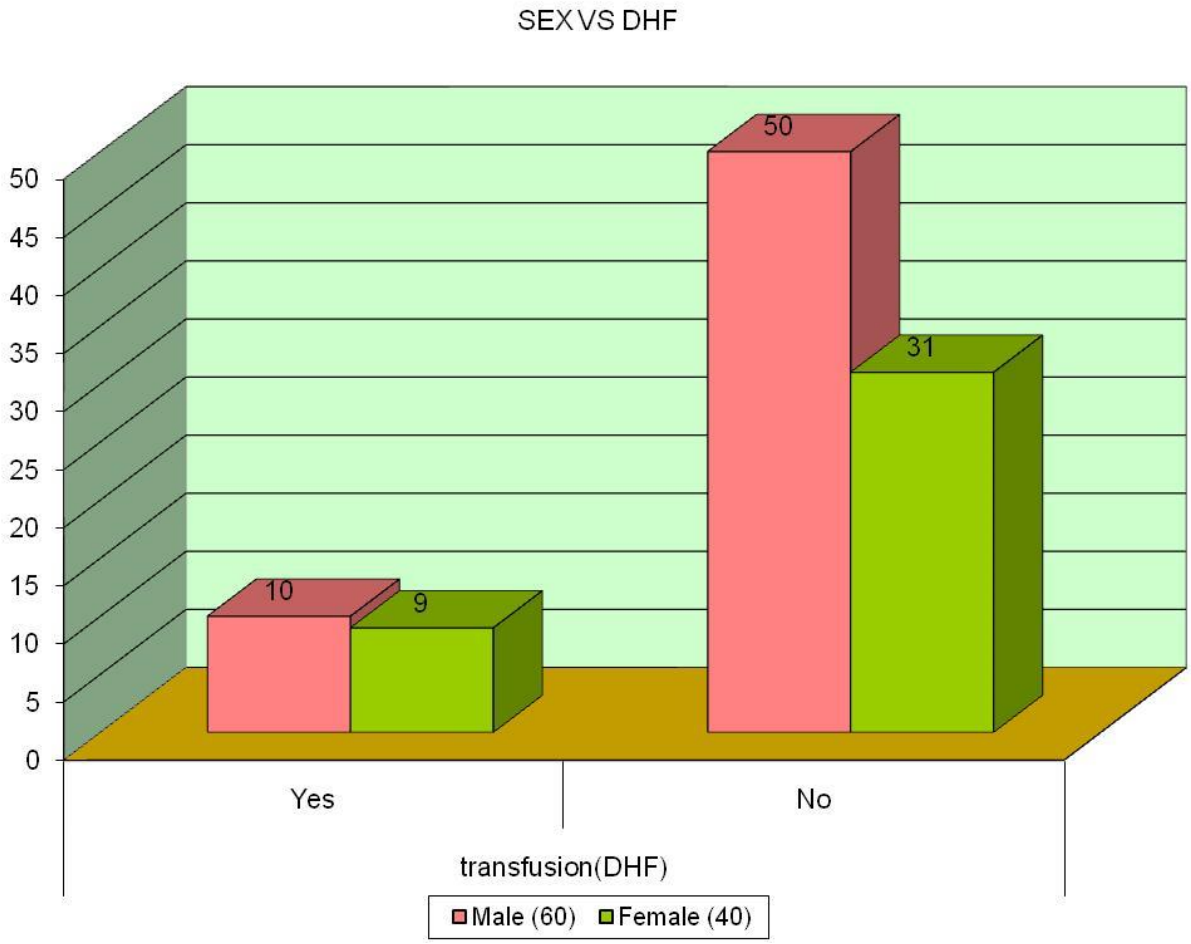
Age vs DSS	DSS	
	Yes	No
<20 (10)	5	5
20-40 (51)	6	45
>40 (39)	10	29
Total	21	79



On seeing the age related incidence of DSS in <20yrs age group it was 50%,in 20-40yrs age group 11.7% and among the >40 yrs group it was 25.6%.

Table 6: Sex distribution among DHF patients

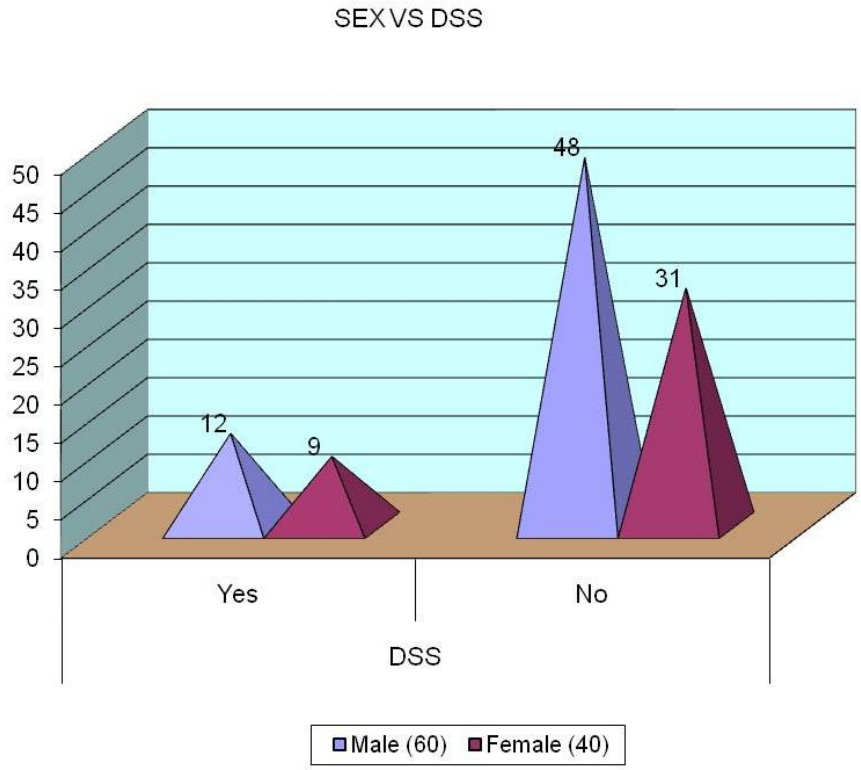
Sex vs DHF	transfusion(DHF)	
	Yes	No
Male (60)	10	50
Female (40)	9	31
Total	19	81



In the sex related distrubtion DHF occurred in 10 men and 19 women. The incidence was 16.6% among men and 21.9% among women.

Table 7:Sex distribution among DSS patients

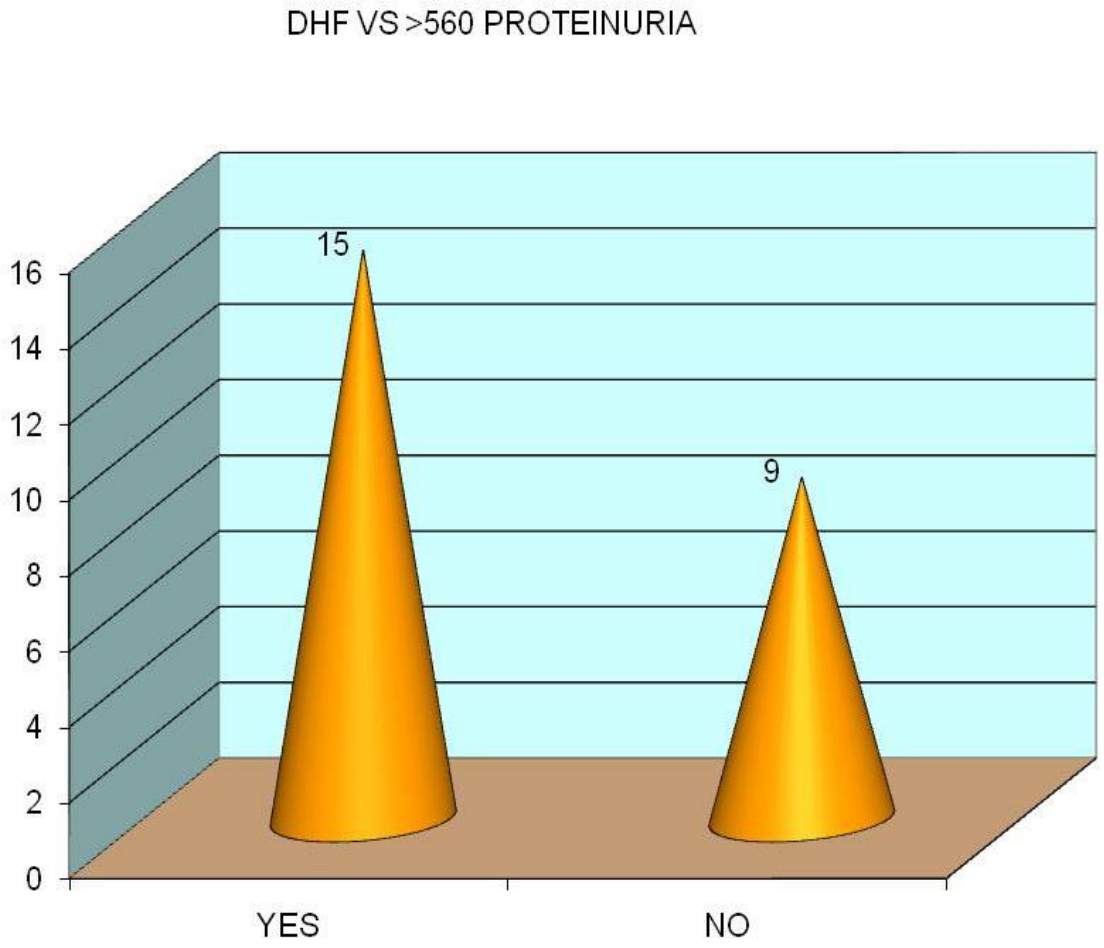
Sex vs DSS	DSS	
	Yes	No
Male (60)	12	48
Female (40)	9	31
Total	21	79



Of the total 21 patients with DSS 12 were male and 9 were female.

Table 8: Patients with proteinuria >560 mg/g with DHF

DHF in patients with proteinuria >560 mg/g	
YES	15
NO	9
TOTAL	24



Among the 24 patients with proteinuria more than 560 mg/g 15 went for DHF and 8 did not have DHF.

Table 9: Platelet count in patients with proteinuria < 560mg/g

platelet count in proteinuria <560mg/g		
Platelet count	Mean	S.D
day -1	93000	22811.693
0	72605	20823.115
1	61118	25111.998
day 2	80934.2	39809.324

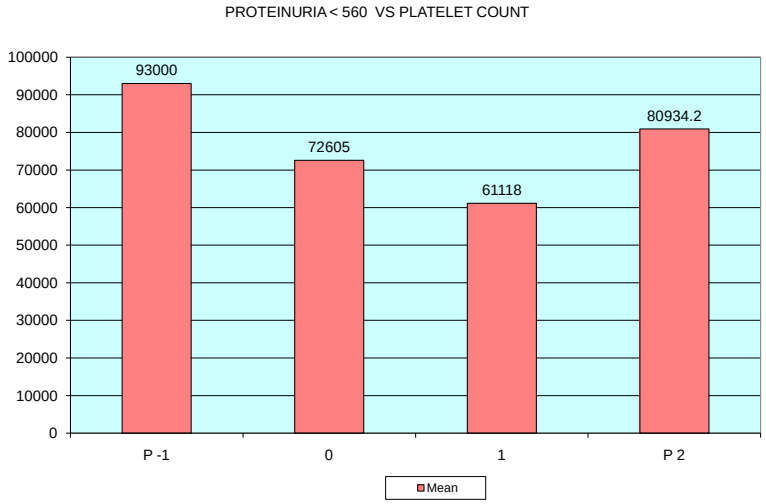


Table 10: Platelet count in patients with proteinuria >560mg/g

proteinuria vs platelet count		
proteinuria vs < 560	Mean	S.D
P -1	93000	22811.693
0	72605	20823.115
1	61118	25111.998
P 2	80934.2	39809.324

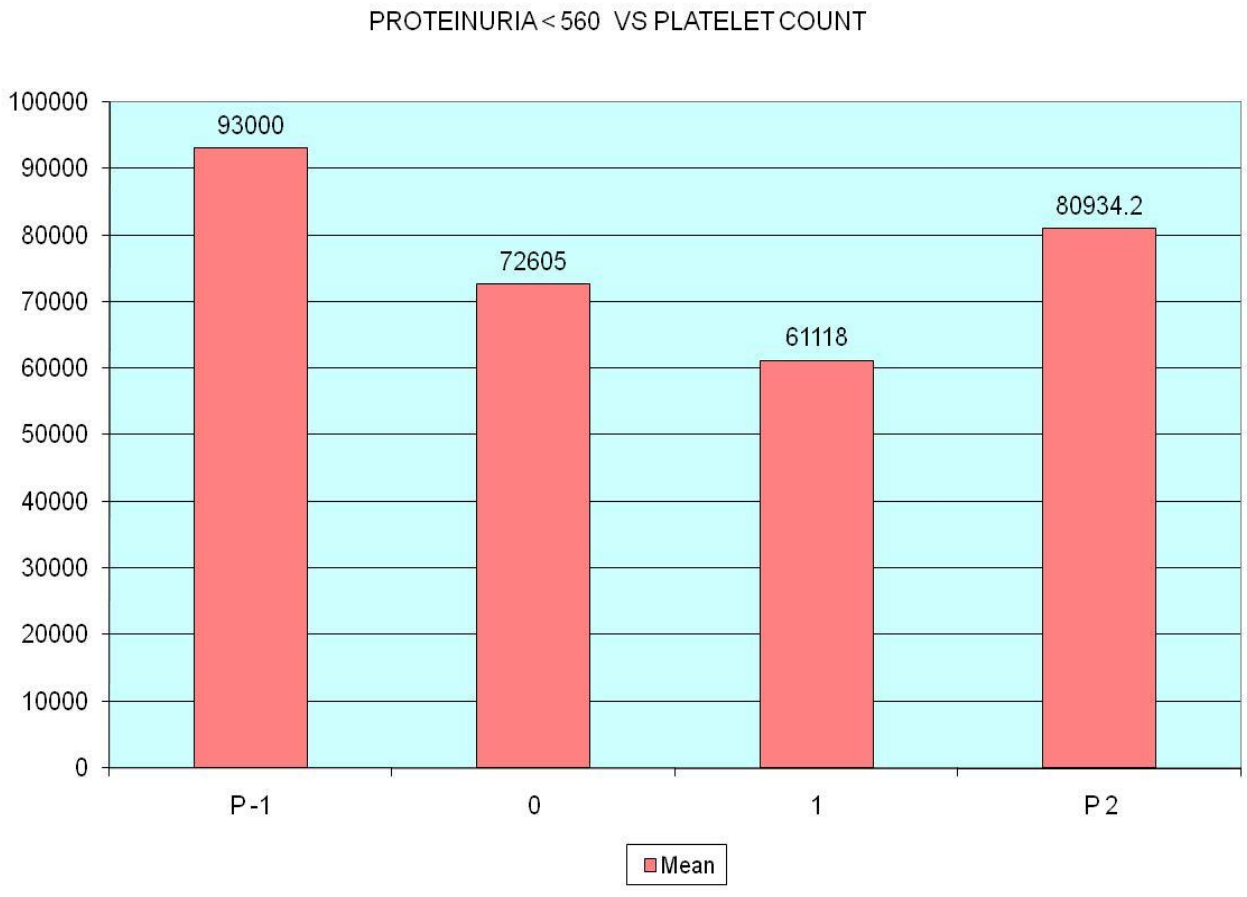
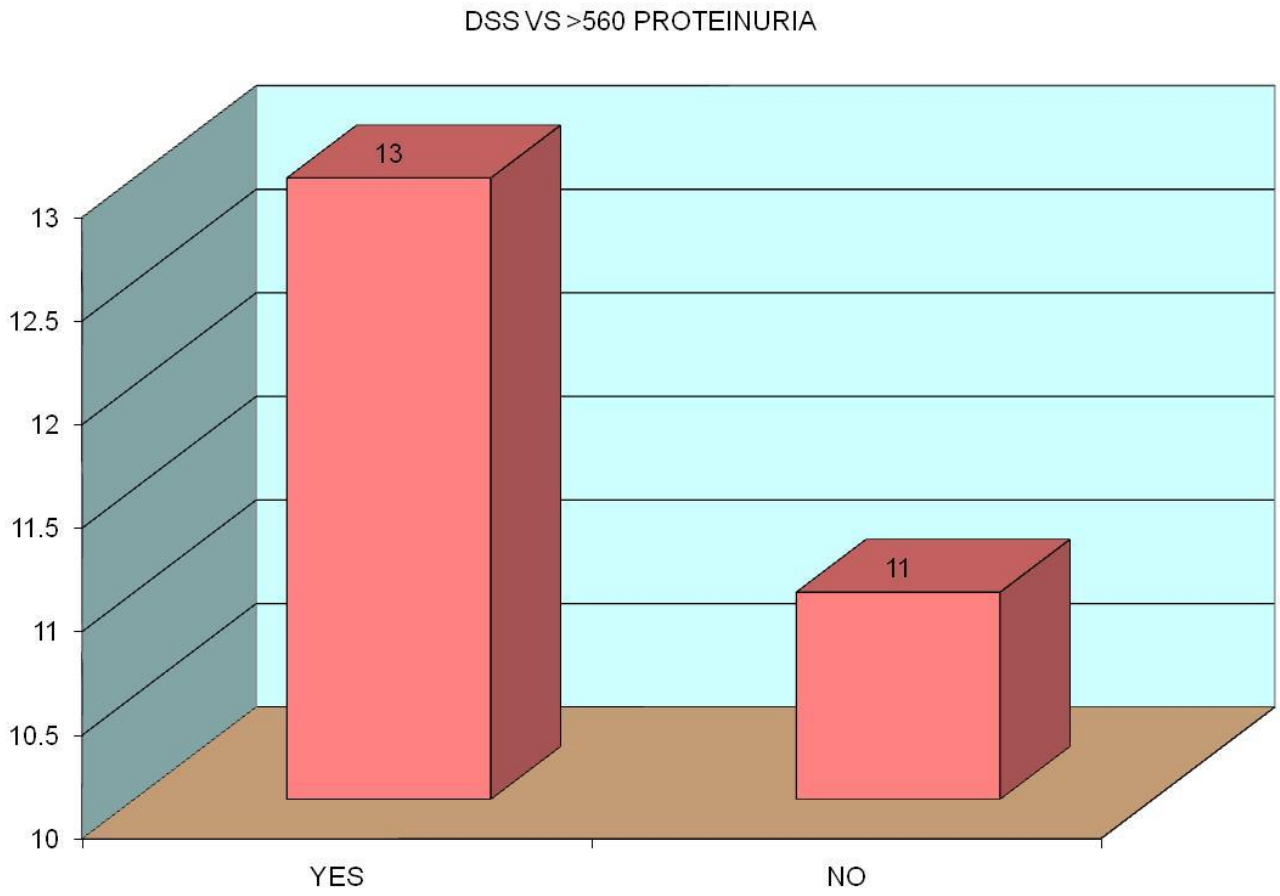


Table 11:DSS in patients with proteinuria >560mg/g

DSS vs in patients with > 560mg/g proteinuria	
YES	13
NO	11
TOTAL	24



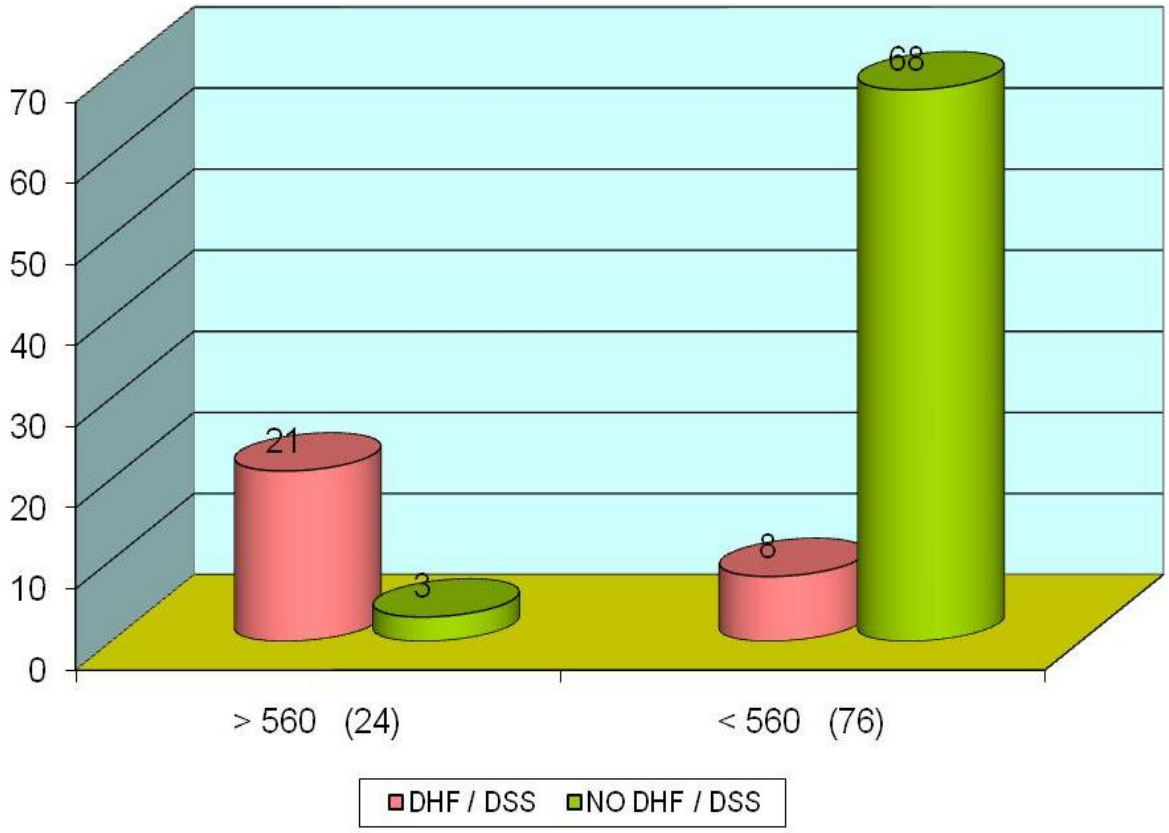
Among the 24 patients with proteinuria more than 560 mg/g 13 patients went for DSS.

Significance of Proteinuria Vs DHF/DSS

Proteinuria	DHF / DSS	NO DHF / DSS
> 560 mg/g (24)	21	3
< 560 mg/g (76)	8	68
p value	<0.001	Significant

In patients with proteinuria > 560 mg/g 21 patients went for DHF/DSS and only three had no progression. Among those with proteinuria less than 560 mg/g only 8 went for DHF/DSS of the total 76. The p value is significant with a confidence interval of 95%.

PROTEINURIA VS DHF/DSS



DISCUSSION

In our study the incidence of dengue hemorrhage fever was 19 % and dengue shock syndrome was 21%. The proteiuria above 560 mg/g occurred in 24 patients among which 87% went for a either dengue hemorrhagic fever or dengue shock syndrome.10 patients had manifestations of both dengue shock syndrome and dengue hemrhagic fever. Urine spot PCR was used a measure for proteinuria because commercial kit for albuminuria did not measure other proteins and quatification with 24 hr urine protein would be cumbersome

The use of urine spot PCR above 560 mg/g had a very significant correlation with development of DHF/DSS. It had a specificity of about 95 %. And a sensitivity of 72.4%. The test also had a positive predictive value of 87.5 % and a negative predictive value of 89.4%.

In a study by vasanwala et al in 2014 which used a cut off value of 29mg/mmol of Spot PCR, it had a sensitivity of 76% and specificity of 77%. When it was combined with platelet

value and age adjustment the sensitivity rose to 90% and specificity to 80%. The higher Spot PCR cut off used in our study could explain for the higher specificity and lower sensitivity seen in our study.

Like in their study the nadir of the platelet values occurred within 2 days around of defervesence. The peak value in the study by vasanwala et al occurred around day of defervesence. So this study was done using the Spot PCR on day of defervesence.

Another study by Andries et al. in Cambodia found that urine diptick test was not efficient to triage the dengue patients. Our study concurs with the study in that the urine proteinuria was higher in patients with DHF and DSS. The andries et al study was done in children. The study also went ahead with urine protein electrophoresis and “Patterns compatible with a tubular proteinuria, a selective glomerular proteinuria and a nonselective glomerular proteinuria, were observed in 51.9%, 22.2% and 14.8% of the samples tested by UPEP, respectively.”

SUMMARY

- Of the total 100 patients, those of age less than 20 constituted were 10, between 20-40 yrs were 51 and more than 40 were 39.
- Among the subjects the total number of men were more accounting 60 and female were 40.
- Among the total 100 patients 24 had urine Spot PCR more than 560 mg/g and 76 had urine spot PCR less 560 mg/g.
- Among the < 20 yrs age group there was 40 % incidence of DHF, among the 20-40 yrs group incidence was 13% and among the >40yrs group it was 20.5%.
- On seeing the age related incidence of DSS in <20yrs age group it was 50%,in 20-40yrs age group 11.7% and among the >40 yrs group it was 25.6%.
- In the sex related distribution DHF occurred in 10 men and 19 women. The incidence was 16.6% among men and 21.9% among women.

- Among the 24 patients with proteinuria more than 560 mg/g 15 went for DHF and 8 did not have DHF
- Among the 24 patients with proteinuria more than 560 mg/g 13 patients went for DSS.
- In patients with proteinuria > 560 mg/g 21 patients went for DHF/DSS and only three had no progression. Among those with proteinuria less than 560 mg/g only 8 went for DHF/DSS of the total 76.
- In our study urine spot PCR >560 mg/g had a specificity of about 95 %. And a sensitivity of 72.4%. The test also had a positive predictive value of 87.5 % and a negative predictive value of 89.4%.

LIMITATIONS

- The nature of proteinuria was not segregated by electrophoresis
- Children in were not included in the study
- Being a tertiary hospital most patients were referred from primary center this may have caused the skew in incidence of the complications
- Renal biopsy was not done to document the glomerulonephritis as it was usually self limiting.

CONCLUSION

There is a need to risk stratify patients with dengue fever, Particularly during the periods of epidemics. The fall in platelet count can predict only to some extent the occurrence of DHF. Urine proteinuria can be used as an additional factor to predict the risk of both DHF and DSS. The peak of urine proteinuria occurs around the day of defervesence. Therefore urine spot PCR can be used as a specific marker for prediction of dengue hemorrhagic fever and Dengue shock syndrome. So patients with higher spot PCR need to shifted to facility with more intensive monitoring than others. The use of urine spot PCR is not recommened as the sensivity of the test based on our study results were low. The test also has a high positive predictive value and also a high predictive value. The dengue algorithm using various clinical biochemical parameters may also be used for predicting the complications.

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ஆராய்ச்சி ஒப்புதல் படிவம்

பெயர்: தேதி:
வயது:
நோயாளிஎண்:

ஆராய்ச்சிசேர்க்கைஎண்:

இந்தஆராய்ச்சியின்விவரங்களும்அதன்நோக்கங்களும் முழுமையாகஎனக்குவிளக்கபட்டது.
எனக்குவிளக்கபட்டவிஷயங்களைநான்புரிந்துகொண்டுஎனது முழுமனதுடன்சம்மதிக்கிறேன்.

இந்தஆராய்ச்சியில்பிறரின்நிர்பந்தமின்றிஎன்சொந்தவிருப்பத்தின்பேரில்தான்பங்குபெறுகிறேன்மற்றும் நான்இந்தஆராய்ச்சியில்இருந்துஎந்தநேரமும்பின்வாங்கலாம்என்றும்அதனால்எந்தபாதிப்பும்எனக்குஏற்படாது என்பதையும்புரிந்துகொண்டேன்.

நான்என்னுடையசுயநினைவுடன்மற்றும்முழுசுதந்திரத்துடன்இந்த
மருத்துவஆராய்ச்சியில்பங்குகொள்ளசம்மதிக்கிறேன்.

MASTER CHART

S.No.	AGE	SEX	SPOT PCR	-1	0	1	2	transfusion(DHF)	DSS
1	17	female	1250	65000	21000	8000	34000	yes	no
2	18	female	103	47000	43000	58000	86000	no	no
3	45	female	2161	24000	21000	15000	11000	no	yes
4	43	female	350	63000	24000	13000	17000	no	no
5	19	female	1091	62000	47000	21000	19000	no	yes
6	20	female	444	48000	24000	21000	23000	no	no
7	21	female	667	48000	40000	26000	45000	no	no
8	18	female	656	58000	39000	15000	9000	yes	yes
9	30	female	643	24000	14000	9000	21000	yes	no
10	19	male	968	56000	20000	11000	19000	yes	yes
11	25	male	1470	78000	39000	21000	19000	yes	no
12	46	male	1435	64000	22000	6000	1000	yes	yes
13	47	male	1144	85000	65000	45000	44000	yes	no
14	18	male	960	96000	92000	95000	111000	no	yes
15	24	male	1605	35000	21000	11000	27000	no	no
16	52	male	1308	66000	35000	21000	41000	yes	yes
17	32	male	1647	113000	56000	19000	26000	yes	no
18	29	male	1501	78000	32000	11000	6000	no	yes
19	52	male	1350	92000	40000	9000	16000	no	no
20	33	male	829	69000	30000	6000	13000	no	yes
21	50	male	1659	91000	63000	23000	9000	yes	no
22	55	male	1337	96000	52000	10000	15000	yes	yes
23	26	male	1792	58000	32000	11000	6000	yes	yes

24	47	male	1567	86000	56000	22000	19000	no	yes
25	34	female	922	63000	54000	25000	9000	yes	no
26	29	female	1260	77000	36000	5000	9000	yes	no
27	52	female	1742	65000	26000	15000	8000	yes	yes
28	54	female	256	70000	56000	30000	48000	no	no
29	26	female	192	105000	90000	88000	112000	no	no
30	35	female	229	62000	50000	36000	60000	no	no
31	20	female	462	62000	36000	21000	43000	no	no
32	36	female	197	78000	70000	98000	152000	no	no
33	52	female	378	125000	90000	140000	210000	no	no
34	48	female	234	63000	51000	78000	130000	no	no
35	47	female	460	61000	42000	25000	39000	no	no
36	34	female	377	130000	96000	82000	98000	no	no
37	28	female	378	98000	89000	72000	83000	no	no
38	50	female	367	68000	37000	22000	56000	no	no
39	43	female	467	76000	70000	52000	58000	no	no
40	39	female	396	64000	58000	54000	69000	no	no
41	44	female	130	94000	85000	80000	120000	no	no
42	27	female	235	70000	52000	35000	68000	no	no
43	27	male	445	71000	60000	62000	88000	no	no
44	25	male	389	92000	90000	96000	152000	no	no
45	40	male	318	77000	86000	94000	148000	no	no
46	25	male	211	109000	87000	46000	98000	no	no
47	55	male	518	76000	32000	16000	24000	no	yes
48	35	male	257	62000	58000	49000	55000	no	no
49	38	male	416	114000	92000	90000	140000	no	no

50	55	male	481	130000	110000	88000	96000	no	no
51	22	male	332	122000	95000	60000	88000	no	no
52	46	male	340	65000	53000	31000	45000	no	yes
53	21	male	123	88000	74000	62000	98000	no	no
54	19	male	322	75000	52000	60000	92000	no	no
55	51	male	394	90000	78000	70000	86000	no	no
56	51	male	204	124000	95000	84000	110000	no	no
57	22	male	381	77000	54000	80000	130000	no	no
58	34	male	300	100000	85000	98000	130000	no	no
59	43	male	385	118000	99000	80000	94000	no	no
60	46	male	310	71000	68000	78000	95000	no	no
61	38	male	460	130000	99000	65000	80000	no	no
62	23	male	415	97000	82000	65000	70000	no	no
63	22	male	508	105000	80000	56000	62000	no	no
64	54	male	492	109000	98000	82000	86000	no	no
65	35	male	311	121000	94000	92000	133000	no	no
66	22	male	246	102000	78000	82000	91000	no	no
67	54	male	348	130000	94000	82000	98000	no	no
68	52	male	187	108000	93000	70000	81000	no	no
69	23	male	166	77000	54000	36000	40000	no	no
70	32	male	118	74000	62000	54000	68000	no	no
71	39	male	425	95000	90000	71000	120000	no	no
72	47	male	152	112000	82000	86000	94000	no	no
73	48	male	130	94000	72000	63000	85000	no	no
74	30	male	372	102000	85000	61000	75000	no	no
75	52	male	150	94000	82000	56000	72000	no	no
76	28	male	541	118000	89000	71000	82000	no	no
77	33	male	366	99000	75000	32000	45000	no	no

78	20	male	211	94000	82000	45000	54000	no	no
79	17	male	281	77000	54000	25000	13000	yes	no
80	19	male	493	101000	79000	45000	30000	no	yes
81	43	male	186	114000	85000	45000	62000	no	no
82	28	male	202	76000	89000	96000	165000	no	no
83	42	male	451	86000	72000	61000	78000	no	no
84	16	male	321	122000	94000	64000	82000	no	no
85	21	female	421	85000	50000	22000	16000	no	yes
86	40	female	166	118000	97000	90000	130000	no	no
87	31	female	518	103000	60000	51000	31000	no	yes
88	52	female	541	62000	54000	31000	12000	yes	yes
89	41	male	211	87000	61000	46000	51000	no	no
90	35	female	508	64000	32000	24000	5000	yes	yes
91	30	female	143	114000	87000	54000	68000	no	no
92	26	female	276	101000	89000	63000	87000	no	no
93	27	female	423	130000	110000	85000	99000	no	no
94	46	female	531	89000	71000	95000	131000	no	no
95	28	female	140	118000	65000	72000	89000	no	no
96	50	female	319	126000	75000	52000	84000	no	no
97	29	female	124	115000	99000	81000	94000	no	no
98	45	male	105	116000	65000	55000	75000	no	no
99	46	male	98	96000	54000	39000	60000	no	no
100	52	female	541	62000	54000	31000	12000	yes	yes

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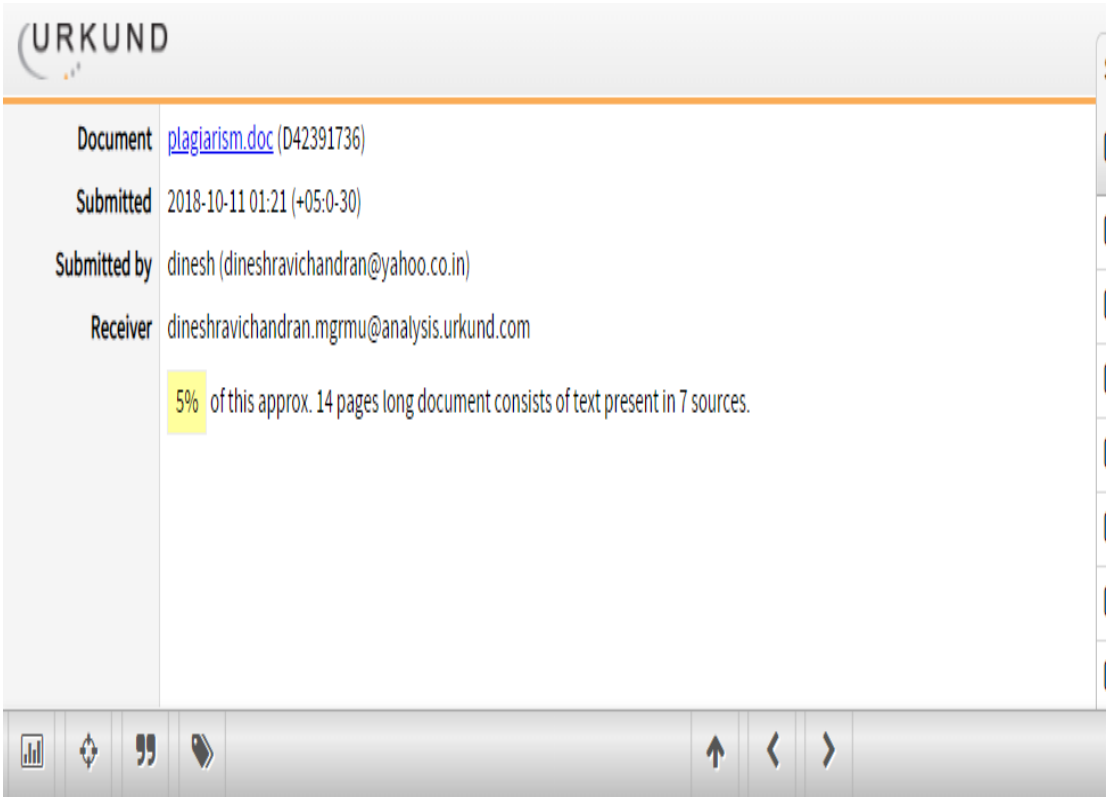

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ETHICAL COMMITTEE APPROVAL LETTER

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ETHICS COMMITTEE CERTIFICATE

Name of the Candidate	:	Dr.R.Dinesh
Course	:	PG in MD., General Medicine
Period of Study	:	2016-2019
College	:	MADURAI MEDICAL COLLEGE
Research Topic	:	Assessment of proteinuria by urine spot protein creatinine ratio for risk prediction of dengue hemorrhagic fever/ dengue shock syndrome in dengue infections.
Ethical Committee as on	:	13.04.18

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

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